

1.0 INTRODUCTION

Malaysia is not a traditional milk producing country and more than half of its population is not traditional milk consumer. However in the last two decades the consumption of milk and milk products in Malaysia has increased by 3 folds, largely as a direct result of changes in lifestyle and surplus income in the medium-high income population group. The production of milk, both by cows and goats has steadily increased during this period. About 75% of Malaysian population (Malay and Chinese) are lactose intolerant. Thus the more digestible processed milk (fermented milk) in the form of yogurt would be more suitable for local consumption. In addition, the longer shelf life and value-added nature of these products would be of advantage in the event of temporary overproduction of milk.

Yogurt is generally recognized as a healthy and multifunctional food. It is a coagulated milk product obtained from the lactic acid fermentation by the action of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Orihara *et al.*, 1992). High levels of live bacterial cultures in yogurt contribute to its nutritional and therapeutic properties. In Malaysia yogurt or “dadih” is consumed occasionally as desserts. Commercial yogurts come in a variety flavour of choices such as corn, chocolate, and strawberry among others.

Fruits are commonly added to enhance the organoleptic properties of yogurt. The presence of these fruits does not only enhance the taste of the yogurt, but also may change the fermentation behaviour and the fermentation byproducts. Therefore, changes in physicochemical, proteolysis, production of exopolysaccharides, organoleptic properties and therapeutical value of yogurt are expected to occur upon the addition of fruit of choice in the present studies i.e. dragon fruits *Hylocereus polyrhizus* and *Hylocereus undatus*. These aspects are also important in determining the eating values of yogurt.

The objectives of the present study are as follows;-

- 1) To determine the effects of *H. polyrhizus* and *H. undatus* on the fermentation of milk by yogurt bacteria by measuring the changes in acid content and pH during fermentation and refrigerated storage.
- 2) To quantitate the amount of exopolysaccharide produced by yogurt bacteria in the presence of *H. polyrhizus* and *H. undatus*.
- 3) To determine the textural properties and eating values of *H. polyrhizus* and *H. undatus* yogurts.
- 4) To evaluate the *in vitro* inhibition of α -amylase and α -glucosida by *H. polyrhizus* and *H. undatus* yogurts.

2.0 LITERATURE REVIEW

2.1 Milk

Milk is a liquid nutrient produced by the mammary glands of female mammals. It provides not only excellent nutrition but also protection against infection for the new-borns (Ivun, *et al.*, 2006) before they are able to digest more solid foods. The cow's milk is most widely used by humans in the world. The average composition of milk is 87.2% water, 3.7% fat, 3.5% protein (mainly casein), 4.9% lactose and 0.7% ash (Heck *et al.*, 2009). These figures also vary by the season of the year, the animal feed content and the breed of the animal. Milk is a good source of calcium and phosphorus (Adolphi *et al.*, 2008) and is recommended to prevent osteoporosis.

2.2 Fermentation of milk

Lactic acid bacteria (LAB) are able to utilize carbon (lactose) and nitrogen (proteins) sources and they convert lactose to lactic acid under anaerobic fermentation of milk. The transportation of lactose into the cell is mediated by the enzyme β -galactosidase, which hydrolyses the lactose inside the LAB cell (Tamime & Robinson, 1985). LAB used as starter cultures for the manufacture of yogurt during fermentation normally consist of a mixture of selected strains of *L. bulgaricus* and *S. thermophilus*.

2.3 Fermented milk

Fermented milk produced by microbial fermentation of milk includes cultured buttermilk, yogurt, acidophilus milk and kefir. Technically, yogurt is defined as milk that has been fermented by *S. thermophilus* and *L. delbrueckii subspp. bulgaricus* under prescribed time and temperature (Vaclavik and Christian, 2008).

2.4 Yogurt

Yogurt evolved empirically centuries ago by allowing nutritionally milk or concentrated milk to sour at warm temperature (40-50°C). Yogurt is a healthy dairy product because it is more digestible than milk and has viable lactic acid bacteria (LAB) (Chen *et al.*, 2009). The lactic acids produced by the LAB in yogurt induce lowering of pH in the gut, which creates an environment less favourable for acid intolerant pathogens (Tamime *et.al.*, 1991). The accumulation of lactic acid also protect yogurt from harmful microorganisms and gives yogurt longer shelf life. Thus, these bacteria foster a healthy colon that reduces the formation of carcinogens (Rafter *et al.*, 2007; Chakraborti, 2011) hence lowering the risk of getting colon cancer (Wollowski *et al.*, 2001).

The consumption of yogurt benefits both infants and elderly persons. As for children it provides balanced source of proteins, fats, carbohydrates and minerals in a texture that is very much to the liking of growing kids. The nutritional constituents of yogurt are derived from a) the milk used, b) fermentation products due to LAB, and c) added ingredients by the manufacturers (Tamime *et al.*, 2007; Vargas *et al.*, 2008). The changes in composition from milk to yogurt are mostly dependent on lactic acid fermentation, which convert the lactose to lactic acid and induce partial digestion of proteins to peptides and amino acids, and fats to fatty acids (Tamime *et al.*, 1991).

2.4.1 Yogurt as a functional food

Functional food is defined as a dietary product that has a health-promoting and/or disease-preventing property beyond the basic nutritional function of supplying nutrients (Korhonen *et al.*, 2009). Yogurt maybe regarded as a functional food primarily because it can serve as a buffer system for the survival of probiotics along the gastrointestinal tract (Viljoen *et al.*, 2001). Several other functional properties functions of yogurt are described as follows:

i) Control of intestinal infections

Yogurt has therapeutic effects on certain gastrointestinal conditions such as lactose intolerance, diarrheal diseases, colon cancer, inflammatory bowel disease, *Helicobacter pylori* infection, allergies (Sovova *et al.*, 2002). Most of the therapeutic effects of yogurt may be explained by the presence of added probiotic bacteria which impart antimicrobial properties (Weng and Wei-Lien, 2008). Probiotic bacteria such as *L. acidophilus* and *Bifidobacterium bifidum* are known to be inhibitory towards many of the commonly known food borne pathogens (Park *et al.*, 2009). The production of organic acids by the probiotics during yogurt fermentation lowers the pH and alters the oxidation-reduction potential in the intestine thus resulting in the antimicrobial action of yogurt (Soghomonyan *et al.*, 2009). Combined with the limited oxygen presence in the intestine, organic acids inhibit especially pathogenic gram-negative bacteria types, e.g coliform bacteria (Dicks and Botes, 2010). Bifidobacteria produce both lactic and acetic acids, but higher amounts of acetic acid exhibits a stronger antagonistic effect against gram negative bacteria than lactic acid (Zouhir *et al.*, 2010).

Probiotic microorganisms may also prevent harmful bacterial colonisation of a habitat by competing more effectively than an invading strain for essential nutrients or adhesion sites. This makes the local environment unfavourable for the growth of the invader

especially when antibacterial substances are also being produced (Fliss *et al.*, 2010). Regular consumption of probiotic bacteria may therefore result in an improved immunological response in human (Dicks and Botes, 2010).

ii) Reducing lactose intolerance

The inability to digest lactose adequately by certain people is due to the absence of β -galactosidase in the intestine and this leads to various degrees of abdominal discomfort (Shaukat *et al.*, 2010). Lactic acid bacteria used in starter culture such as *L. acidophilus* and *B. bifidum* are responsible for the production of β -galactosidase. This enzyme hydrolyses lactose, which results in increased tolerance for dairy products (Sanchez *et al.*, 2009). However, improved digestion of lactose was not caused only by hydrolysis of the lactose prior to consumption. The beneficial effect was shown to have occurred in the digestive tract after consumption of milk containing *L. acidophilus* (Shaukat *et al.*, 2000). Thus, the continued utilisation of lactose within the gastrointestinal tract depends on the survival of the lactobacilli in that environment.

iii) Discouraging vaginal infections

Candida or “yeast” vaginal infections are common problems for women especially those with diabetes. *L. acidophilus*, a strain of friendly bacteria, is an integral part of normal vaginal flora. Lactobacilli prevent overgrowth of unfriendly bacteria and Candida via the formation of lactic acid which act like a natural antibiotic (Sandine, 1979; Bruno, 2009). Besides, these friendly bacteria also compete with other organisms for the utilisation of glucose with the added advantage that the production of lactic acid and hydrogen peroxide by the lactobacilli helps to maintain acidic pH needed for healthy vaginal flora to thrive (Sandine, 1979; Ahmad *et al.*, 2007).

iv) Reducing serum cholesterol level

Fermented milk is considered instrumental in reducing serum cholesterol since late 1960's (Gilliland *et al.*, 1989). *L. acidophilus* deconjugates bile acids into free acids, which are excreted more rapidly from the intestinal tract than the conjugated bile acids. Since free bile salts are excreted from the body, the need to synthesize new bile acids from blood cholesterol can reduce the total cholesterol concentration in the body (Gilliland *et al.*, 1977; Lee *et al.*, 2009). Another hypothesis is that reduction of cholesterol may also be due to co-precipitation of cholesterol with deconjugated bile salts at lower pH values as a result of lactic acid production by the bacteria (Viljoen *et al.*, 2001; Lee *et al.*, 2009).

v) Anticarcinogenic activity

The antitumour action of probiotics is attributed to the inhibition of carcinogens and/or procarcinogens, inhibition of bacteria that convert procarcinogens to carcinogens (Gilliland *et al.*, 1989; Gursoy and Kinik, 2010), activation of the host's immune system and/or reduction of intestinal pH to reduce microbial activity (Rasic, 1983; Mishra *et al.*, 2008). The intake of yogurt and fermented milks containing probiotic bacteria which inhibited tumour formation and proliferation in animals has made yogurt gain popularity as functional food (Kailasapathy and Rybka, 1997; Jha *et al.*, 2008).

Yogurt excelled milk in terms of better digestibility, therefore improved nutrition absorption. Lactase-deficient individuals tend to have impaired calcium absorption. Thus, consumption of yogurt provides them with high calcium food to prevent osteoporosis (Wynckel *et al.*, 1991; Parra *et al.*, 2007). In conclusion, yogurt is a functional food that puts together milk's nutritional characteristics along with numerous promising therapeutic benefits.

2.5 Probiotics

Probiotics are the living microbial feed supplements added to the diet which provide beneficial effects on the host (only for animal) by improving their intestinal microflora balance (Fuller, 1989; Sanders, 2009; Wallace *et al.*, 2011). Common probiotics used to achieve these effects include various species of the genera *Bifidobacterium* and *Lactobacillus* such as *L. acidophilus*, *L. casei*, *L. plantarum*, *Bifidobacteria lactis*, *B. bifidum*, *B. infantis*, *B.* and many more. These microbes provide the health benefits by improving lactose digestion by producing an enzyme which helps digest lactose (Kim and Gilliland, 1983; Aryana and McGrew, 2007), reducing side-effects of antibiotics by reestablishing the “healthy” intestinal bacteria very quickly after antibiotic treatment (Lidbeck, 1995; Granato *et al.*, 2010), and preventing intestinal infections by production of organic acids and other antibacterial agents and competitive colonization (Gilliland & Speck, 1977; Kamiya, 2011). Other benefits of probiotics include preventing cancers especially colorectal cancer (Comanne *et al.*, 2005; Ishikawa *et al.*, 2005; Thomas, 2010), improving the immune system (Hatcher and Lambrecht, 1993; Nomoto, 2010) and lowering cholesterol (Gilliland & Walker, 1990; Kamiya, 2011).

Consumption of probiotic bacteria via food products is an ideal way to re-establish the intestinal microflora balance. Some yogurt products are reformulated to include live strains of *Lactobacillus acidophilus* and species of *Bifidobacterium* (known as AB-cultures) in addition to conventional yogurt organisms, *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* (Lourens-Hattingh and Viljoen, 2001). This type of yogurt, also known as bio-yogurt, is a potential vehicle by which consumers can obtain probiotic bacteria.

A bacterial culture must be conformed to certain requirements before it can be used as a dietary adjunct (Chou *et al.*, 1992; Park *et al.*, 2010). The culture must be a normal

inhabitant of the human intestinal tract, survives passage through the upper digestive tract in larger numbers, be capable of filling an ecological niche, and have beneficial effects when in the intestine (Gilliland *et al.*, 1989; Dicks and Botes, 2010). The strain must be resistant to bile salts present in the lower intestine, acidic gastric conditions (pH1-4), digestive enzymes present in the intestine (lysozyme) and toxic metabolites produced during digestion in order to survive (Hoier, 1992; Mortazavian *et al.*, 2007). The bacteria used in traditional yogurt fermentation, *L. bulgaricus* and *S. thermophilus* are not bile acid resistant and thus they do not belong to the indigenous intestinal flora (Shah, 2007).

The addition of yogurt bacteria (*S. thermophilus* and *L. bulgaricus*) into starter culture is a usual practice in order to reduce the fermentation time. This is because the probiotic bacteria (*L. acidophilus* and *Bifidobacterium spp*) lack proteolytic activity (Weerkamp *et al.*, 1993; El-Dieb *et al.*, 2010) and therefore grow slowly in milk. *L. bulgaricus* produces essential amino acids owing to its proteolytic nature and the symbiotic relationship between *L. bulgaricus* and *S. thermophilus* is well established i.e. the former microorganism produces amino nitrogen for the latter organism. *S. thermophilus* acting as an oxygen scavenger, creates an anaerobic environment and may enhance growth and survival of *Bifidobacterium* when used together in starter culture (Rybka, 1994; Shah 2007).

Bio-yogurts are considered to have functional properties because of the probiotic bacteria incorporated into the regular fermentation cultures provide therapeutic benefits. These include improving lactose digestion by producing an enzyme which helps digest lactose (Kim & Gilliland, 1983; Ranadheera *et al.*, 2010; Goldin, 2011), reducing side effects of antibiotics by re-establishing the “healthy” intestinal bacteria after antibiotic treatment (Lidbeck, 1995; Granato *et al.*, 2010), preventing intestinal infections by competitive colonization, organic acids production and other antibacterial agents (Aryana *et*

al., 2007). Other benefits of probiotics include preventing cancers (Kailasapathy and Rybka, 1997), improving the immune system (Hatcher & Lambrecht, 1993) and lowering cholesterol (Gilliland and Walker, 1990). The main therapeutic benefits attributed to consumption of probiotics are summarized in Table 2.1

Table 2.1: Benefits and potential therapeutical applications

Beneficial effects:

- i) Maintenance of normal intestinal microflora.
- ii) Enhancement of the immune system.
- iii) Reduction of lactose-intolerance.
- iv) Reduction of serum cholesterol levels.
- v) Anticarcinogenic activity.
- vi) Improved nutritional value of foods.

Therapeutic applications

- i) Prevention of urogenital infection.
- ii) Alleviation of constipation.
- iii) Protection against traveller's diarrhoea.
- iv) Prevention of infantile diarrhoea.
- v) Reduction of antibiotic-induced diarrhoea.
- vi) Prevention of hypercholesterolaemia.
- vii) Protection against colon/bladder cancer.
- viii) Prevention of osteoporosis.

Adequate numbers of viable probiotics cells, namely the 'therapeutically minimum bacteria count' need to be consumed regularly, are considered to be at least 10^6 cfu/ml (Kurmann and Rasic, 1991) at the time of yogurt production. This is to ensure that the optimum number of bacteria is reasonably high during shelf life and at the time of

consumption (Viljoen *et al.*, 2001;). The survival of probiotic bacteria in fermented dairy bio-products depends on many factors such as the strain used, interaction between species present, culture conditions, chemical composition of the fermentation medium (e.g carbohydrate source), final acidity, milk solid content, availability of nutrients, growth promoters and inhibitors, concentration of sugars (osmotic pressure), dissolved oxygen (especially for strictly anaerobic *Bifidobacterium* sp), level of inoculation, incubation temperature, fermentation time and storage temperature (Kailasapathy *et al.*, 2008).

2.6 Extracellular polysaccharides (Exopolysaccharides)

Several bacteria are known to synthesize exopolysaccharides (EPS). These EPS occur in two forms depending on their location. They are called as either capsular polysaccharides whereby the polymer is closely associated with the cell surface or slime polysaccharides in which they are loosely associated with the cell surface (De Vuyst *et al.*, 2001). EPS produced by the bacteria are thought to protect the cells against desiccation, toxic compounds, bacteriophages, osmotic stress, and permit adhesion to solid surfaces and biofilm formation (De Vuyst and Degeest, 1999). In the food industry, these polymers find useful application as biothickeners because of their stabilizing, emulsifying or gelling properties (De Vuyst and Degeest, 1999; Jafarei and Ebrahimi, 2011).

Bacterial polysaccharides can also be differentiated by their chemical compositions. Homopolysaccharides are composed of typically one monosaccharide (which is mainly glucose or fructose), whereas heteropolysaccharides are composed of at least two different monosaccharides (Leivers *et al.*, 2009). Other residues such as sn-glycerol-3-phosphate, *N*-acetyl-amino sugars, phosphate, and acetyl groups can also be found in heteropolysaccharides (Laws *et al.*, 2001). In addition to other criteria such as molecular

mass and conformation, EPS characteristics and amounts can be influenced by several factors such as location and composition of the medium (carbon and nitrogen sources), as well as incubation conditions like temperature, pH, time, etc (Cerning, 1990; Laws *et al.*, 2009).

2.6.1 Exopolysaccharides in yogurt

EPSs produced by LAB are increasingly used in food industry because the bacteria are food grade organisms and they are considered as generally-recognized-as safe (GRAS) (Kitazawa, *et al.*, 2003). LAB produces EPSs which are potentially useful as additives to improve texture and viscosity of natural fermented milk products and to prevent syneresis (Kitazawa, *et al.*, 2003). Moreover, EPS produced by lactic acid bacteria may confer health benefits to the consumer in the form of immunostimulatory (Hosono, *et al.*, 1997) and antitumoral activities in addition to the activation of macrophages and lymphocytes (via the presence of phosphate groups in EPS) (Kitazawa, *et al.*, 2003). Investigation on LAB production are largely carried out on thermophilic (e.g *L. delbrueckii* subsp. *bulgaricus*, *L. helveticus* and *S. thermophilus*) or mesophilic (e.g *L. acidophilus*, *Lactococcus lactis*, *L. rhamnosus*, and *L. casei*) bacteria. The latter is mainly of economic concern in the dairy industry (Cerning *et al.*, 1992).

. The carbohydrates in dairy microbial exopolysaccharides are extremely diverse and may consist of D-glucose, D-galactose, and D-mannose, 6-deoxyhexoses, L-fucose, and L-rhamnose (Cerning, 1990; Cerning and Marshall, 1999; De Vuyst and Degeest, 1999; Ricciardi and Clementi, 2000; Sikkema and Oba, 1998). The natural biothickening effects of EPS are important in improving the rheological properties of yogurt. It acts as physical stabilizers (Faber, 2000) to bind water and limit syneresis (Rapaille and Vanhemelrijck, 1994). LAB are able to synthesize and secrete EPS into their environment such as milk

(Cerning, 1990; Cerning and Marshall, 1999; De Vuyst and Degeest, 1999; Ricciardi and Clementi, 2000; Sikkema and Oba, 1998) and are therefore important particularly for improving the texture, mouth-feel, taste perception and stability of drinking yogurt, cheese, fermented cream and milk-based desserts (Bouzar, Cerning, and Desmazeaud, 1997; Cerning, 1995; Christiansen, Madeira, and Edelstein, 1999; Crescenzi, 1995). The function of EPS as stabilizer depends on the composition, structure and interaction of the EPS with milk constituents, mainly ions and proteins (Sebastiani and Zelger, 1998; Skriver *et al.*, 1999; Skriver *et al.*, 1993).

EPS affects two important rheological characteristics of yogurt i.e. viscosity and elasticity. Viscosity represents the property of a material to resist deformation. In the context of fermented dairy products, this attribute can be described as slimy fluid. On the other hand, elasticity is the property of a material to recover after a deformation occurred. This attribute corresponds to a firm body and gum-like fermented milk product (Sebastiani and Zelger, 1998; Skriver *et al.*, 1999; Skriver *et al.*, 1993). Both viscosity and elasticity are important for the organoleptic quality of a product and for its appealing appearance and pleasant mouth feel (Sebastiani and Zelger, 1998; Skriver *et al.*, 1999; Skriver *et al.*, 1993). The texture of the product may be summarised due to the presence of a bio-thickener in the aqueous phase (serum), the existence of a protein gel (mainly constituted of caseins), the interaction between proteins and polysaccharides, the presence of bacterial cells and filaments of EPS bound to them, the binding of hydration water that reduces the amount of free water molecules and the consequent increment of the EPS apparent concentration in the serum phase (Sebastiani and Zelger, 1998; Skriver *et al.*, 1999; Skriver *et al.*, 1993).

The benefits of EPS may be obtained at extremely low concentrations. This is evident in yogurt whereby the appealing visual appearance (gloss) of a product, minimal syneresis, a

creamy and firm texture, and pleasant mouth-feel are associated with concentration of EPS of 490-3100 mg/L (De Vuyst et al., 2003; Duboc and Mollet, 2001). Since the production of one kind of EPS may not satisfy all texture specifications, the production of several EPS by one or several starter cultures may be formulated to precisely create the desirable texture of an end product and to match the consumer preferences that can vary from one country to another (Duboc & Miller, 2001).

2.7 Syneresis

Syneresis is the collection of whey on the surface of yogurt as a result of the formation of curd. This occurs due to the sudden removal of the hydrophilic micropeptides which causes an imbalance in intermolecular forces (Amatayakul *et al.*, 2006). The bonds between hydrophobic sites start to develop and are enforced by calcium bonds which forms as the micelles start to leave the structure (Hallen *et al.*, 2010). This process is usually referred to as the phase of coagulation and syneresis. Primary rennet action (first phase) occur after the splitting of the bond between residue 105 and 106 in the κ -casein molecule, whereas the phase of coagulation and syneresis are referred to as the secondary phase (Grachev *et al.*, 2008; Costa et al., 2011).

Milk gels can be formed by enzymatic action (by rennet as in natural cheese), acidification (by starter bacteria as in yogurt), heat (as in whey protein gelation) or by combination of several of these techniques (Lucey, 2001). Syneresis is an essential part in cheese making because the dewatering of curd particles which is necessary to achieve appropriate moisture content in cheese whereas whey separation is a major defect when it occurs on the surface of set-style yogurt. Syneresis can be reduced or eliminated by either increasing the level of milk solid up to 15% (Tamime and Deeth, 1980; Shah, 2003) or using

stabilizers (e.g starch, gelatin, and vegetables gum) or exopolysaccharides (EPS) producing stater cultures (Tamime and Deeth, 1980; Shah, 2003).

In rennet-induced milk gels the susceptibility to syneresis is associated with a high value for the loss tangent at long time scales (Van Vliet *et al.*, 1991) which is related to the propensity of the network to rearrange after gel formation. On the other hand, yogurt's milk gel is formed by gradual acidification with a lactic acid bacteria starter, which has some problems of whey separation, or syneresis, with a change of temperature or physical impact (Kuraishi *et al.*, 1996). Meanwhile, the reduction of syneresis may be caused by the effect of transglutaminase on the pore size of the milk gels, in which as pore size reduces, the protein network will result in lower syneresis (Lorenzen *et al.*, 2002).

2.8 Proteolysis

Proteolysis is the breakdown of large and complex proteins into the smaller and simple peptides. Various functional and bioactive peptides and amino acids are released due to the proteolytic activity of enzyme proteinase and peptidases (Serra *et al.*, 2009) which are related especially to lactic acid bacteria (Ramchandran and Shah, 2009). Proteinase activity can be expressed in terms of the amount of peptides produced. Different level of proteolysis by the enzymatic activities occurred at different temperature and pH condition. The physiological and biotechnological significance of these peptides in dairy products were reviewed by Gobbetti, (2002).

Starter cultures are used in yogurt production because the concentrations of free amino acids and peptides are very low in milk. The degradation of milk protein (caseins) by proteinases and peptidases from LAB which yields small peptides and free amino acids (Kunji *et al.*, 1996) results in the breakdown of the protein network (Pripp *et al.*, 2006).

Proteolysis in yogurt can have some bearings on the texture, taste and flavour development during fermentation and storage period. This is because the liberated peptides and free amino acids used in bacterial growth (Kunji *et al.*, 1996; Nielsen *et al.*, 2009) also results in the formation of volatile flavour compounds (Marilley and Casey, 2004). Some of the peptides can taste bitter (Lemieux and Simard, 1992) or delicious (Yamasaki and Maekawa, 1978) whereas amino acids can taste sweet, bitter or broth-like (Mulder, 1952).

Proteolysis is initiated by a single cell-wall-bound extracellular proteinase which can be either chromosomally or plasmid-encoded. Most of the LAB strains contain such extracellular proteinases for the production of peptides and amino acids which are subsequently taken into the cells by the transporters in the membrane cells (Jeanguenin *et al.*, 2009). Following uptake, the peptides are degraded intracellularly by a variety of enzymes (Kunji *et al.*, 1996) classified as endopeptidases, aminopeptidases, di-/tri-peptidases, and proline-specific peptidases into amino acids. The activities of other enzymes such as deaminases, decarboxylases, transaminases and lyases result in the formation of α -keto acids that can be converted into aldehydes by decarboxylation and subsequently into alcohols or carboxylic acids by dehydrogenation (Kunji *et al.*, 1996; Cadwallader and Singh, 2009).

2.8.1 O-phthaldehyde in the evaluation of proteolysis

The extent to which the functional properties of a protein may be altered by hydrolysis is very much dependant on the degree to which the protein has been hydrolysed (Fitzgerald *et al.*, 2003). Obtaining a value for the actual number of peptide bonds cleaved during the reaction, or degree of hydrolysis (DH) is a useful way of monitoring the extent of protein degradation. DH is defined as the proportion of cleaved peptide bonds in a protein hydrolysate (Rutherford, 2010).

Ninhydrin, Trinitrobenzenesulphonic acid (TNBS), and Folin-Ciocalteu phenol reagent are widely used to assess proteolysis of protein. This is despite the relatively high blank readings obtained (Stein *et al.*, 1954). Ninhydrin is perhaps the most widely used reagent, but heating and cooling steps are required (Church *et al.*, 1983). The methods by TNBS also suffer from high blank readings (usually from contamination of reagent by picric acid) (Satake *et al.*, 1960). The Folin Ciocalteu reagent, although specific for tyrosine and tryptophan, exhibits interference from a large number of compounds that compromise its accuracy (Weinstein *et al.*, 1976).

OPA has very high sensitivity detection reagent of amines, peptides, and amino acids. The compound o-phthalaldehyde (OPA) in conjunction with reduced sulphydryl group reacts with primary amines to form fluorescent moieties (Roth, 1971). When reacted with primary amines in the presence of mercapethanol, OPA yields an intense blue coloured fluorescent product that has a maximum wavelength of excitation of 340 nm and emission at 455 nm (Ogden and Foldi., 1987). The OPA spectrophotometric assay provides a rapid, convenient and sensitive procedure for determination of proteolysis in milk and milk proteins. This assay is able to react with 18 of the 20 common amino acids, measure protein concentration as low as 50ng/ml and quantify accurately the number of peptide bonds released during hydrolysis of a protein substrate (Fitzgerald *et al.*, 2003). Specific characteristics that make the OPA assay a desirable method for monitoring hydrolysis are: i) the OPA reagent is soluble and stable in aqueous solutions, ii) the reaction with primary amines proceeds essentially to completion within seconds at ambient temperature, and iii) a single reagent solution serves both to inhibit proteolytic activities and develop the reaction colour (Church *et al.*, 1983).

A major concern of the assay of soluble proteinases is the inactivation of the proteolytic enzyme at the appropriate time. Inclusion of 1% SDS in the OPA reagent not only serves to denature the protein substrate to ensure complete reaction of available amino groups but also to terminate the proteolytic action in most cases. Assay of a mixture of soluble trypsin chymotrypsin with either β -lactoglobulin, α -lactalbumin, or whole casein demonstrates the convenience of the OPA spectrophotometric assay for monitoring the hydrolysis of milk proteins (Church *et al.*, 1983).

2.9 α -Amylase and α -glucosidase enzyme

Amylase is a digestive enzyme classified as a saccharidase (an enzyme that cleaves polysaccharides). It is a main constituent of pancreatic juice and saliva, needed for the breakdown of long-chain carbohydrates (such as starch) into smaller units (Lin *et al.*, 2009). The primary function of the enzyme amylase is to break down starches in food to smaller molecules so that these can be broken down further to much smaller molecules which ready to be used by the body. α -Amylase hydrolyze alpha-1, 4-glycosidic linkages, randomly yielding dextrans, oligosaccharides and monosaccharides or maltose and maltotriose (Gutiérrez, *et al.*, 1990; Tortora, 2008). This enzyme also hydrolyses glycogen, the reserve carbohydrates in animals, when the blood glucose levels are low (Gutiérrez, *et al.*, 1990).

α -Glucosidase (α -D-glucohydrolase EC. 3.2.1.20), is a group of membrane-bound enzymes including maltase, isomaltase, and glucoamylase which are located at the epithelium of the small intestine (Cheeseman *et al.*, 1997; Rita, 2010) and they function as the key enzymes of carbohydrate digestion (Caspary, 1978). The soluble form of the enzyme is a monomer with a molecular weight of 98000 Da (Scaman *et al.*, 2002). It specifically hydrolyses the α -glucopyranoside bond, thereby releasing a α -D-glucose from the non-reducing end of sugar.

2.9.1 Inhibition of pancreatic α -amylase and intestinal α -glucosidase

Hydrolysis of dietary carbohydrates such as starches is the major source of glucose in the blood glucose. The enterocytes of the small intestine can only absorb monosaccharides such as glucose and fructose from the diet (Elsenhans and Caspary, 1987). The cellular balance of carbohydrate and lipid metabolism is affected by improper glucose metabolism (Chang *et al.*, 2002) and this is caused by elevated postprandial blood glucose levels. Hyperglycemia, a condition characterized by an abnormal postprandial increase of blood glucose level, is linked to the onset of type 2 insulin-dependent diabetes mellitus and associated cardiovascular complications including hypertension (Tiwari and Rao, 2002). Inhibitors of these α -glucosidase and α -amylase were shown to delay carbohydrate digestion and prolong overall carbohydrate digestion in time (Wild *et al.*, 2004). This causes a reduction in the rate of glucose absorption and consequently suppressing the incident of rapid plasma glucose rise (Wild *et al.*, 2004).

A main drawback of using drugs such as acarbose and miglitol to inhibit α -amylase or α -glucosidase is the side effects such as abnormal distention, flatulence, and possibly diarrhea (Shetty *et al.*, 2006). Such adverse effects can be caused by the excessive inhibition of pancreatic α -amylase resulting in the abnormal bacterial fermentation of undigested carbohydrates in the colon (Shetty *et al.*, 2006). In this regard, natural α -amylase and α -glucosidase inhibitors offer an attractive therapeutic approach to the treatment of postprandial hyperglycemia by ultimately slowing glucose release from starch (Shetty *et al.*, 2006), but not to completely inhibit starch breakdown, thus causing minimal side effects. Due to this, dairy products (McCue *et al.*, 2005) and herbal medicines (Marles and Fansworth, 1995; Alarcon-Aguilara *et al.*, 1998) with mild α -glucosidase inhibitory activity have been studied for applications in diabetes treatment.

2.10 Antioxidant activity

2.10.1 Oxidative stress and antioxidants

The oxygen consumption inherent in energy production in cell growth leads to the generation of a series of free radicals of oxygen which contribute to the phenomenon known as “oxidative stress”. The stress increases the formation of superoxide radical and hydrogen peroxide that directly promotes cellular damage (Berset *et al.*, 1994). These oxygen-related products can also interact in the presence of suitable transition metal catalysts to form highly toxic hydroxyl radicals and other oxidizing species (Tsai *et al.*, 2002). As a result NADH, GSH, and ATP are depleted, whereas calcium ion is increased and these induce cell damage instrumental in the forming of diseases such as arteriosclerosis, cancer and ischaemia (Tsai *et al.*, 2002).

Hyperglycemia in particular triggers the generation of free radicals and the oxidative stress (Brownlee, 2005) and this has damaging effects in capillaries of endothelial cells in the retina, mesangial cells in the renal glomerulus and neuron cells in the peripheral nerves (Brownlee, 2005). Therefore, it is essential to regenerate critical cellular antioxidant responses to manage cellular redox status to minimise or even prevent these diabetic complications (Shetty *et al.*, 2006). Herbs and fruits have been widely studied for their antioxidative effects (Vattern *et al.*, 2005). Other foods such as yogurt also exert certain level of antioxidant activity (Shetty *et al.*, 2006).

Antioxidant is a molecule that is capable of inhibiting the oxidation of other molecules (Seis and Helmut, 1997). These antioxidants scavenge free radicals by inhibiting initiation and breaking chain propagation or suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide and quenching superoxide and singlet oxygen (Shi, Noguchi and Niki, 2001). Substances with antioxidation activities can prevent or delay

oxidative damage of lipids, proteins and nucleic acids by reactive oxygen species such as reactive free radicals (superoxide, hydroxyl, peroxy, alkoxy) and non-radicals (hydrogen peroxide, hypochlorous). Fruits are rich with antioxidants and these can lower the incidence of degenerative diseases such as cancer, arthritis, arteriosclerosis, heart disease, inflammation, brain dysfunction and acceleration of the ageing process (Feskanich *et al.*, 2000; Gordon, 1996; Halliwell, 1996). The most abundant antioxidants in fruits are polyphenols whereas Vitamin C, Vitamins A, B and E and carotenoids are present to a lesser extent in some fruits. These polyphenols, most of which are flavonoids, are present mainly in ester and glycoside forms (Fleuriet and Macheix, 2003). The fruits of *Hylocereus cacti*, also known as red pitaya or pitahaya have gained popularity not only because of their red-purple colour and economic value as food products, but also for their antioxidative activity attributed to the betacyanin contents (Wybraniec and Mizrahi, 2002).

2.10.2 Determination of antioxidant capacity

Phenolic and polyphenolic compounds constitute the main class of natural antioxidants present in plants, foods and beverages and are usually quantified colorimetrically using the Folin-Ciocalteu reagent (Vinson *et al.*, 1998). There are several different methods to determine the antioxidant capacity such as the Trolox equivalent antioxidant capacity (TEAC), DPPH (using 2,2-diphenyl-1-picrylhydrazyl radical), and ferric reducing antioxidant power (FRAP) methods. These methods differ in terms of their assay principle and experimental conditions. The DPPH assay uses the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) to test the ability of plant compounds to act as free radical scavengers or hydrogen donors. The DPPH method is used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the

sample (Miller *et al.*, 2001). A measure of total antioxidant capacity provides more insight in the understanding of the functional properties of foods.

The 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay has been widely used to test the free radical scavenging ability of various natural products (Williams *et al.*, 1995) and is accepted as a compound model for free radicals originating in lipids (Singh *et al.*, 2007). This method allows one to determine exclusively the intrinsic ability of a sample to donate hydrogen atoms or electrons to this reactive species in a homogeneous system (Wang *et al.*, 2006). The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H (Wang *et al.*, 2006) by the reaction. A blue-violet colour changes gradually to green and yellow and a decrease in absorbance at 517 nm was monitored during the reaction.

2.11 Phenolic phytochemicals

Phenolic phytochemicals are secondary metabolites of plants and they constitute one of the most abundant groups of natural metabolites synthesized by plants in order to protect themselves from biological and environmental stresses (Shetty *et al.*, 2005). In addition to their high antioxidant activities (Vattern *et al.*, 2005), the phenolic compounds possess a wide spectrum of biochemical activities including anti-diabetic, anti-hypertension activity as well as antimicrobial activities (Shetty *et al.*, 1997).

Polyphenols are classified based on their carbon skeleton into non-flavonoid (stilbenes, hydroxycinnamic acids, and benzoic acids) or flavanoid (flavanols, flavones, flavonols, isoflavones, anthocyanin and catechins) compounds. There are more than 4,000 different flavonoids being identified (Lanzotti *et al.*, 2006) and their reactivity is due to the acidic character of the phenolic functions and to the nucleophilic character of the benzene

rings. Flavonoids are implicated in the management of many chronic oxidation-linked diseases such as diabetes and cardiovascular disease (Atanassova, 2005).

The formulation of antioxidant-related nutrition requires information about phenolic and flavonoid composition in herbs-based foods (Atanassova, 2005). Total phenols and polyphenols are usually quantified by employing Folin-Ciocalteu's reagent (FCR), where gallic acid is used as a standard. FCR is non-specific to phenolic compounds but it reacts with phenolic compounds only under basic conditions (Camara *et al.*, 2007). Even with the undefined chemical nature of FCR, the total phenols assay by FCR is convenient, simple and reproducible and it is commonly accepted assay and routinely used in investigation on dietary antioxidants (Huang *et al.*, 2005).

Many of the natural components of plant materials have antioxidant activity. Polyphenols are secondary plant metabolites and confer on fruits and vegetables both desirable and undesirable food qualities. Polyphenols account for the majority of antioxidant activity in plants. The antioxidant properties of phenolic compounds are mainly because of their redox potential, which allow them to act as reducing agents, hydrogen donators, metal chelators and singlet oxygen quenchers (Rice-Evan *et al.*, 1996). The degree of glycosylation significantly affects the antioxidant properties of the compounds, for example, aglycons of quercetin and myricetin were more active than their glycosides (Marchand, 2002).

Flavonoids are naturally occurring phenolic compounds which largely include anthoxanthins (flavones, flavonols, flavanones, flavanols, chalcones and isoflavones), anthocyanins, leucoanthoxanthins and flavonoidal alkaloids (Houghton, 2002). These compounds are found in a variety of plant materials (Kong *et al.*, 2003) and they possess antioxidant properties as demonstrated *in vitro* and *in vivo*. The flavonoids contain a number of phenolic hydroxyl groups attached to ring structures, which confer the antioxidant activity. Catechins

and their epimers serve as powerful antioxidants for directly eliminating superoxide anion radicals (Chen and Chan, 1996). Proanthocyanidins from grape seeds are apparently responsible for the action on the cardiovascular system (Pekić *et al.*, 1997). Kaempferol 3-O- α -rhamnoside from *Licania licaniaeflora* exhibited DPPH radical scavenging activity and quercetin derivatives from this plant showed strong antiradical activity (Braca *et al.*, 2002). Epicatechin, epigallocatechin, epicatechin gallate and procyanidin B1 and B2 from grape seed extract showed strong DPPH radical scavenging activity (Guendez *et al.*, 2005).

The antioxidant activity of phenolic acids and their esters depends on the number of hydroxyl groups in the molecule and the activity can be strengthened by steric hindrance. The electron withdrawing properties of the carboxylate group in benzoic acids has a negative influence on the H- donating abilities of the hydroxy benzoates. Thus, hydroxylated cinnamates are more effective than benzoate counterparts (Rice-Evans *et al.*, 1996). Neochlorogenic acid and cryptochlorogenic acid isolated from prunes can scavenge superoxide anion radicals and inhibit oxidation of the methyl linoleate system (Nakatani *et al.*, 2000).

2.12 Dragon fruit (Pitaya)

Dragon fruit (*Hylocereus sp*), also called Pitaya or Pitahaya (Canto, 2000), are consumed largely in Asian countries such as Taiwan, Vietnam, Thailand, the Philippines, Sri Lanka and Malaysia. They originated from Mexico and Central as well as South America (Mizrahi *et al.*, 1997). This fruit comes from the family Cactaceae and its typical pH is 5. Dragon fruit is covered by the skins which look like “scales” (Raveh *et al.*, 1993) and thus the naming of “dragon fruit” (Wu *et al.*, 2006).

Hylocereus has a creamy pulp and a delicate aroma. Dragon fruit with white flesh is called *Hylocereus undatus*, whereas dragon fruit with red flesh is called *Hylocereus polyrhizus*. *Hylocereus costaricensis* on the other hand has red skin with deep purple flesh (Arcadio, 1986; Barbeau, 1990; Mizrahi and Nerd, 1999; Le Bellec *et al.*, 2006).



(a)



(b)

Figure 2.0: (a) *Hylocereus undatus* (white-flesh); and (b) *Hylocereus polyrhizus* (red-flesh)

Dragon fruits are nutritious as they are good source of beta-carotene, lycopene and vitamin E, with average concentrations of 1.4, 3.4 and 0.26 $\mu\text{g}/100\text{ g}$ edible portion respectively (Charoensiri *et al.*, 2009). Typical nutritional values per 100 g of fresh weight dragon fruit are fiber (0.9 g), calcium (8.8 g), fat (0.61 g), carotene (0.012 g), niacin (0.430 mg), phosphorus (36.1 mg), ascorbic acid (8.1 mg), protein (0.229 g), water (83.0 g), ashes (0.68g), iron (0.65 mg), and riboflavin (0.045 mg). In particular red-skinned pitayas contains high concentration of Vitamin C (9.0 mg) compared to white pitayas (~8.1mg; Palande, 2010). In general *H. undatus* has more soluble solid content than *H. polyrhizus* (Palande, 2010).

Studies by Wichienchot *et al.*, (2010) showed that sugars of white- and red-flesh dragon fruit consisted mostly of glucose, fructose and some oligosaccharides (Figure 2.1).

Glucose (41-89%), fructose (32-64%) and sucrose (2.8-7.5%) are the major soluble sugars in the flesh of dragon fruit (Ming et al., 1997). Glucose concentration *H. undatus* (353 ± 0.7 g/kg) is lower than *H. polyrhizus* (401 ± 1.27 g/kg) ($p \leq 0.05$). In contrast, fructose content in *H. undatus* (238 ± 0.84 g/kg) is higher than that in *H. polyrhizus* (158 ± 0.32 g/kg) ($p \leq 0.05$). Oligosaccharides content in red (89.6 ± 0.76 g/kg) and white (86.2 ± 0.93 g/kg) flesh dragon fruit are similar.

The essential linoleic acid and linolenic acid from dragon fruit seeds contribute to a significant percentage (48%, 1.5%) of the unsaturated fatty acids of the seed oil extract (Ariffin et al., 2008). Both pitaya varieties show two oleic acid isomers. Essential fatty acids are important prebiotic as they cannot be synthesized and act as substrates in animal metabolism (Ariffin et al., 2008).

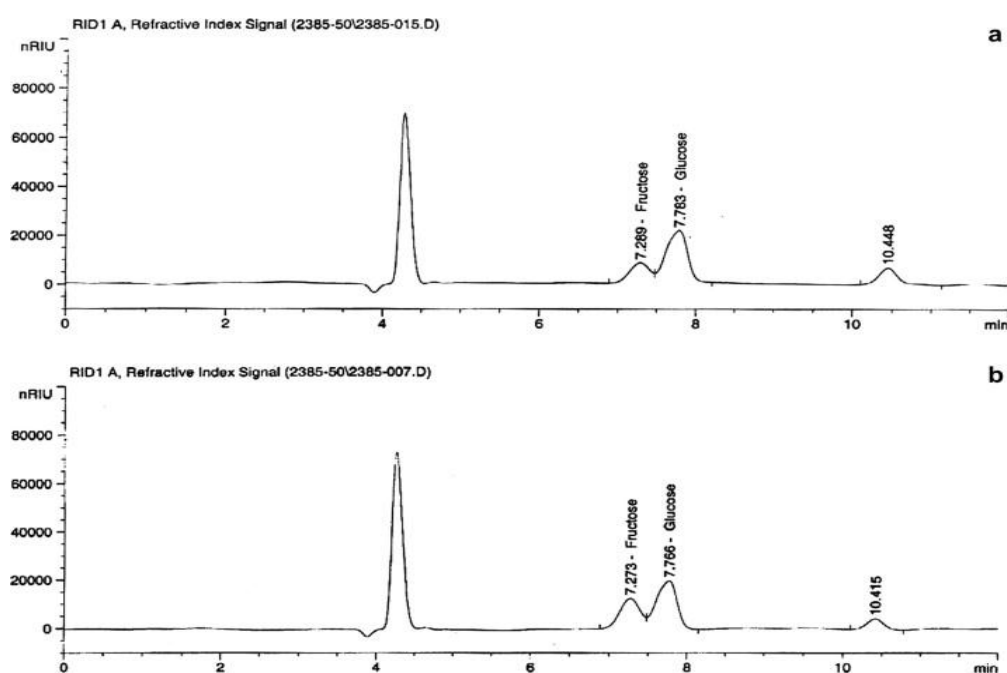


Figure 2.1: Sugar composition of (a) white-flesh; and (b) red-flesh dragon fruit extracts, analyzed by (Wichienchot et al., 2010)

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Fruits

White and red dragon fruits (*Hylocereus polyrhizus* and *Hylocereus undatus* respectively) were purchased from local store. These fruits were purchased within five days after harvest after which the skins can easily become damaged and the fruits are considered spoiled.

3.1.2 Yogurt bacteria

Bacteria used in the making of probiotic yogurt were a mixture of Nn Yogurt Mix (sachet) and Biolife Advance Multiblend Probiotic Live and Active Cultures (capsule). The former consist of *Lactobacillus Acidophilus* LA-5, *Bifidobacterium* Bb-12, *Lactobacillus casei* LC-01 and *Streptococcus thermophilus* Th-4 in the ratio of 4:4:1:1 whereas the latter consist of *Lactobacillus acidophilus*, *L. delbrueckii ssp. bulgaricus*, *L. casei*, *L. rhamnosus*, *Bifidobacterium bifidum*, *B. infantis*, and *B. longum* in equal amount.

3.2 Methods

3.2.1 Apparatus

Glasswares and reusable plastics were washed thoroughly prior to sterilization by autoclaving before being used. These include beakers (50ml, 100ml, 250ml, 500ml, 1000ml and 2000ml), measuring cylinder (10ml, 25ml, 50ml, and 100ml), Erlenmeyer Flask (50ml and 100ml) and test tubes.

3.2.2 Preparation of starter culture

Pasteurized full cream milk was heated to 41°C. The bacteria mixtures (see section 3.1.2) were resuspended in 100 ml of milk followed by mixing in a total volume of 1 L of preheated (41°C) milk. Incubation was carried out at 41°C until the pH (monitored by Cyber Scan 510 pH meter) reached 4.5. The yogurt formed was stored at 4°C and used as starter culture within 3 days.

3.2.3 Preparation of yogurt

Plain yogurt was made by initially mixing 10ml of starter culture with 90ml of preheated milk. Red or white dragon fruits-yogurts at varying amount of inclusion (10%, 20%, 30%) w/w were made by adding 10g, 20g, or 30g of gently mashed fruit into 80, 70, or 60ml of preheated milk respectively followed by the addition of 10ml starter culture. Total milk solid content for the yogurt was corrected by adding 0.2g of milk powder for every 10ml of mashed fruit used. The milk-starter culture or fruit-milk-starter culture mixtures were incubated at 41°C until the pH reached 4.5. The yogurts were then stored at 4°C until required for analysis.

3.2.4 Preparation of yogurt water extract

Samples of yogurt (10g) were mixed with 2.5ml distilled water and the yogurt pH was adjusted to 4 using 1M HCl. The yogurt was then incubated at 45 °C for 10 minutes followed by centrifugation (10000rpm, 20 minutes, 4°C). The supernatant was harvested and the pH was adjusted to 7 using NaOH prior to a second centrifugation (10000rpm, 20 minutes, 4°C). The harvested supernatants were kept at 4°C and were analysed within 4 days of extraction.

3.2.5 pH and titratable acid measurement

The changes of pH in yogurt during fermentation were monitored (Cyber scan 510 pH meter) at every 30 minutes interval. Samples of yogurt (1ml) were mixed with 1 ml of distilled water prior to pH meter reading. The monitoring of pH reading was carried out for each treatment until the pH reading reached constant values.

Titrateable acid (TA) was determined by titration using 0.1N NaOH. To do this, yogurt sample (1ml) was initially transferred into an Ehrlenmeyer flask containing 9 ml distilled water followed by the addition of a few drops of 0.1% phenolphthalein. NaOH (0.1N) was titrated drop by drop into the solution and the solution was thoroughly mixed. The process was repeated until the phenolphthalein changed to pink colour lasting at least 30 seconds. Titrateable acid (% lactic equivalent) was calculated as follows:

$$\text{Titrateable acid (\%)} = \frac{10 \times V_{\text{NaOH}} \times 0.009 \times 0.1}{W} \times 100\%$$

W

Where 10 = Dilution factor

W = Weight of sample for titration

V_{NaOH} = Volume of NaOH used to neutralize the lactic acid

0.1 = Normality of NaOH

3.2.6 Syneresis measurement

Yogurt syneresis (the release of whey) was determined by centrifugation according to Keogh and O' Kennedy (1998). In brief, yogurt samples (20g) carefully scooped from the containers were weighed (correct to four decimal places) followed by centrifugation (640g, 20min, 4°C) and the clear supernatant was harvested and weighed. Syneresis was calculated according to the following equation:

$$\text{Syneresis (\%)} = \frac{\text{Weight of supernatant (g)}}{\text{Weight of yogurt sample (g)}} \times 100\%$$

3.2.7 The total phenolic content assay

The total phenolic content (TPC) was determined according to Shetty *et al.*, (1995). Yogurt water extract (1.0ml) was transferred into a test tube and mixed with 1ml of 95% ethanol and 5ml of distilled water. Folin-Ciocalteu reagent 0.5ml of 50% (v/v) was added to each sample and the mixture was vortexed briefly. The test tubes were left to stand at room temperature and after 5 minutes, 1ml of 5% Na₂CO₃ was added and the reaction mixture was allowed to stand for 60 minutes. The absorbance was read at 725 nm and the values were converted to total phenolics, expressed in micrograms equivalents of gallic acid per gram sample (µgGAE/g). Gallic acid (50 - 250 mg/l) was used as standards.

3.2.8 Antioxidant activity by 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition assay

Antioxidant activity was determined as described by Shetty *et al.*, (1995). Yogurt water extract (250µl) was added into 3ml of 60 µM DPPH in ethanol. The decrease in absorbance (A_{extract}) was monitored at 517 nm until a constant reading was obtained. The readings were compared with the control (A_{control}) which contained distilled water (250 µl)

instead of yogurt water extract. The inhibition of DPPH oxidation activity was calculated as follows:

$$\text{Inhibition (\%)} = \frac{(A_{\text{control}} - A_{\text{extract}})}{A_{\text{control}}} \times 100\%$$

3.2.9 Proteolysis assessment in yogurt

3.2.9.1 Preparation of OPA reagent

The OPA reagent was prepared as described by Goodno *et al.*, (1981). The OPA solution was made by combining the following reagents: 25 ml of 100mM sodium tetraborate, 2.5 ml of 20% (w/w) SDS, 40 mg of OPA (dissolved in 1 ml of methanol) and 100 µl of β-mercaptoethanol. The volume was then made up to 50 ml in a volumetric flask. This reagent was prepared fresh and used within 2 hours of preparation. OPA reagent is light-sensitive, and thus must be protected from light during preparation and running of the assay.

3.2.9.2 OPA assay

A small aliquot of yogurt extract (usually 10 to 50 µl containing 5 to 100 µg proteins) was added directly to 1.0 ml of OPA reagent in a 1.5 ml cuvette. The solution was mixed briefly by inversion and incubated at room temperature (25 °C) for 2 minutes. The absorbance readings were determined at 340 nm. Standard peptide concentrations (0.4- 1.1 mg/ml) were prepared using tryptone stock solution (1.5mg/ml). These standards were prepared fresh every time OPA assay was carried out.

3.2.10 Inhibition of α -glucosidase activity

3.2.10.1 Preparation of reagents

i) α -Glucosidase enzyme solution

α -Glucosidase (1000 U) was dissolved in 100 ml of 0.1 M potassium phosphate buffer (pH6.90). Aliquotes of 500 μ l were made and these were stored at -20 °C. Each ampoule contained 5U/500 μ l of α -glucosidase enzyme solution.

ii) 0.1 M potassium phosphate buffer (pH6.90)

The following two solutions were prepared separately.

i) Distilled water (200ml) was added to 9.11g K_2HPO_4

ii) Distilled water (200ml) was added to 6.49g KH_2PO_4

It is important to ensure that the salts were properly dissolved. Both solutions (i) and (ii) were then mixed thoroughly together followed by the addition of 400 ml of distilled water. The pH was adjusted to 6.90 by adding either K_2HPO_4 as base or KH_2PO_4 as acid. Finally, the solution was brought up to a final volume of 1000 ml by adding distilled water in a volumetric flask. The buffer prepared was stored at 4 °C and used within 2 weeks.

iii) 5mM p-nitrophenyl- α -D-glucopyranoside substrate solution

Potassium phosphate buffer (0.1M, pH 6.90) was slowly added into 5mM p-nitrophenyl- α -D-glucopyranoside under continuous stirring to ensure thorough mixing. This solution was prepared fresh prior to assay.

3.2.10.2 α -Glucosidase inhibition assay

The α -glucosidase inhibition assay was carried out using the method described by Shetty *et al.*, (2006). The reaction mixture which contains 500 μ l of sample extract and 1ml of 0.1 M potassium phosphate buffer (pH 6.9) containing α -glucosidase solution

(1.0U/ml) was initially incubated in water bath at 37 °C for 10 minutes. After 10 minutes, 500µl of 5mM p-nitrophenyl –α-D-glucopyranoside solution in 0.1 ml potassium phosphate buffer (pH 6.90) was added to each tube at time interval of 30 seconds. The mixtures were further incubated at 37 °C for 10 minutes. Absorbance readings at 405nm (Shimadzu mini 1240) were recorded before and after the 10 minutes incubation. The difference (Δ) in the readings was compared to control which consists of 500µl of phosphate buffer solution in place of the yogurt water extract. The inhibition of enzyme activity was calculated as follows:

$$\text{Inhibition (\%)} = \left(\frac{\Delta \text{Absorbance}_{\text{control}} - \Delta \text{Absorbance}_{\text{extract}}}{\Delta \text{Absorbance}_{\text{control}}} \right) \times 100\%$$

Inhibition was expressed as the concentration of inhibitory compound that inhibits 50% of α-glucosidase activity (IC₅₀), assuming that the activity of the blank was 100%. IC₅₀ was essentially obtained after subjecting the percentage of α-glucosidase inhibition against 3 different doses of yogurt extracts to linear estimation of 50% α-glucosidase inhibition. Besides 500µl, the 2 other volumes of extract tested were 250µl and 125µl. The volume of extract was made up to 500 µl by adding 250µl and 375µl buffer respectively.

3.2.10.3 Preliminary studies on the inhibition of enzymes by yogurt water extract

Preliminary studies were carried out to determine the optimum condition for the α-glucosidase assay in order to get consistent results, particularly in the presence of dragon fruit.

i) The optimum enzyme concentration and incubation time

The assay system was as described in section 3.2.10.2. The enzyme solutions used were neat (1mU/ml), 2 times diluted, and 4 times diluted. The absorbance readings were

taken for every minute which is from 0 minute to the 25th minute to allow the reaction to reach completion.

ii) The optimum dilution factor of yogurt extract

The assay system was as described in section 3.2.10.2. The yogurt water extract used was neat (1mg/ml), 2 times diluted, and 4 times diluted. The absorbance readings were taken every minute which is from 0 minute to the 25th minute.

3.2.11 α -Amylase inhibition assay

3.2.11.1 Reagent preparation

i) α -Amylase enzyme solution

Porcine pancreatic α -amylase (EC 3.2.1.1) was purchased from Sigma Chemical Co (USA). A unit of enzyme was defined as the liberation of 1.0mg of maltose from starch after 3 minutes incubation (20°C) at pH 7 (Apostolidis et al., 2006). The enzyme concentration used in the present assay was 0.5 mg/ml. The lyophilised enzyme powder was dissolved in pre-chilled 0.02M sodium phosphate buffer, pH 6.9 with 0.006M sodium chloride yielding a clear to hazy solution. This is due to the presence of enzyme carriers, lactose which are partially soluble in chilled buffer. The enzyme solution was prepared fresh prior to analysis.

ii) Sodium phosphate buffer (0.02M, pH 6.9 with 0.006 M sodium chloride)

The following solutions (A, B, and C) were prepared separately. Each solution was stirred thoroughly to ensure complete dissolution of added salts. Solution A was prepared by adding 200 ml distilled water to 1.582g of Na₂HPO₄. Solution B was prepared by adding 200ml distilled water to 1.062g of NaH₂PO₄, and solution C was prepared by adding 100ml distilled water to 0.3506g of NaCl. All 3 solutions were then mixed together followed by the

the addition of 400ml of distilled water. The pH was adjusted to 6.90 if necessary by adding either Na_2HPO_4 as base or NaH_2PO_4 as acid. Finally, the solution was brought up to a final volume of 1000ml in a volumetric flask. The buffer prepared was stored at 25 °C and used within 14 days.

iii) Starch solution

Starch (1.0g) was dissolved in 100 ml of sodium phosphate buffer prepared (see section (3.2.11.1(i))). The mixture was subjected to heating (approximately 90 °C) with constant stirring to facilitate the dissolution of starch into the buffer. The starch solution was then cooled to room temperature and the final volume was brought up to 100 ml (if necessary) by adding distilled water. The starch solution can be stored at room temperature (25°C) and used within 2 weeks.

iv) Dinitrosalicylic acid (DNSA) reagent

The modified DNSA reagent was initially prepared by dissolving 1.40g NaOH in 70 ml of DNSA. Another component of modified DNSA reagent, the 18.2% (w/v) potassium sodium tartarate, also known as Rochelle salts, was to be prepared separately using distilled water. DNSA was prepared fresh prior to assay. Precautionary steps were taken to exclude carbon dioxide as this gas may interfere the stability of the reagent. DNSA reagent was also kept protected from light by wrapping the flask with aluminium foil.

3.2.11.2 α -Amylase inhibition assay

The α -amylase inhibition assay was carried out as described by Shetty *et al.*, (2006). Yogurt water extract (500 μl) was added to 0.02M sodium phosphate buffer, pH 6.9 with 0.006 M sodium chloride containing 0.5mg/ml α -amylase solution. The mixture was initially incubated at 25°C for 10 minutes followed by the addition of 500 μl of a 1% starch

solution in 0.02M sodium phosphate buffer, pH 6.9 with 0.006M sodium chloride at predetermined time intervals. The reaction mixtures were then incubated for another 10 minutes after which the reaction was terminated with 1.0 ml of DNSA colour reagent. The test tubes were then incubated in boiling water bath for 7 minutes followed by the addition of 1.0ml of 18.2% tartarate solution. The reaction mixture, upon cooling to room temperature, was then diluted by the addition of 10 ml of distilled water. Absorbance reading was taken at 540 nm and the enzyme activity inhibition was calculated as follows:

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{extract}})}{\text{Absorbance}_{\text{control}}} \times 100\%$$

α -Amylase IC₅₀ (the concentration of inhibitory compound that inhibits 50% of α -amylase activity, assuming that the activity of the blank was 100%) was obtained by plotting a graph of inhibition percentage against 3 different doses of yogurt extracts. Besides 500 μ l yogurt water extract, the other 2 volumes tested were 250 μ l and 125 μ l. These were diluted with 250 μ l and 375 μ l buffer respectively.

3.2.11.3 Preliminary experiments on optimisation of α -amylase assay conditions

Preliminary studies were carried out to determine the optimum condition for the α -amylase assay in order to get consistent results.

i) The optimal composition of DNSA reagent

The dinitrosalicylic acid (DNSA) reagent, developed by Sumner and Sisler (1929) for the determination of reducing sugar, is composed of DNSA, Potassium-sodium tartarate (Rochelle salt), phenol, bisulfite and sodium hydroxide. Minor changes to the assay condition were made. For the following reasons (Summer, 1924; Summer, 1925): tartarate is introduced to prevent the reagent from dissolving oxygen; phenol to increase the amount of colour produced; and bisulfite to stabilize the colour obtained in the presence of phenol. The

alkali is required for the reducing action of glucose on dinitrosalicylic acid. The major defect in the test is in the loss of part of the reducing sugar being analyzed. This has been repeatedly observed in the laboratory. The present study was carried out to investigate the different factors which might cause the loss of reducing sugar.

The original DNSA reagent developed by Summer and Sisler (1921) was initially tested. The composition of Summer's reagent contained 0.63% DNSA, 18.2% tartarate, 0.5% phenol, 0.5% sodium bisulfite, and 2.14% NaOH. Subsequently, a modified DNSA reagent developed by Miller (1959) was tested. This reagent contained 1% DNSA, 0.2% phenol, 0.15% sodium bisulfite, and 2.14% NaOH, with 18.2% tartarate, all of which were prepared separately. The assay condition was further tested without the presence of phenol and sodium bisulfite as they are not the essential components. At the same time, several concentrations of DNS (1%, 1.5%, 2%, and 2.5%) were tested.

ii) The optimum dilution factor of yogurt extracts

Five dragon fruit-yogurt extracts was serially diluted (2 fold dilution) from neat to yield 2X (0.5), 4X (0.25) and finally 8X (0.125) dilution. Yogurt extracts with 1.5X (0.67) dilution factor was prepared by adding 1.0 ml of distilled water to 0.5 ml yogurt water extracts. The experiment was carried out as described in section 3.2.11.2. Comparison in the percentage of inhibition in α -amylase activity was made by plotting all the fruit-yogurts onto the same graph.

iii) The optimum incubation time

An experiment was carried out (as described in section 3.2.11.2) to determine the optimum incubation time (reaction time tested were from 0 – 15 minutes) upon the addition of starch (substrate) to the mixture of enzyme and extracts.

iv) The optimum boiling time

The optimum boiling time (from 0 - 10 minutes) after the addition of DNSA reagent into the reaction mixtures was determined because this step is crucial for the colour development for subsequent absorbance measurement. The experiment was carried out as described in section 3.2.11.2

3.2.12 Production of exopolysaccharides in dragon fruits yogurts

3.2.12.1 Extraction, purification and quantification of exopolysaccharides

The extraction and purification of exopolysaccharides (EPS) in yogurt was carried out as described by Cerning et al., (1988). Yogurt sample (50ml) was added to 50 ml of distilled water prior to mixing with 4 ml of 20 % TCA. The mixture was then centrifuged (3500 g, 30 minutes, 4°C) and the supernatant pH was adjusted to 6.8 using 40 % (w/v) NaOH. The supernatant was then boiled at 100°C for 30 minutes prior to a second centrifugation (3500 g, 4°C, 30 minutes). The supernatant was then mixed with 50 ml cold ethanol prior to being kept overnight at 4°C after which the supernatant was centrifuged again (3500g, 4°C, 30 minutes). The supernatant was then discarded and the pellet (EPS precipitate) was resuspended in 10 ml distilled water under sonication for 1 hour at room temperature. The dissolved EPS was dialyzed in a membrane (13000 Da cut-off) for 2 weeks. The water was changed twice a week. Phenol-sulphuric acid method (Dubois *et al.*, 1956) was used to quantify the yield of EPS.

3.2.12.2 Phenol-sulphuric acid assay

The phenol-sulphuric acid assay was carried out using the method described by Dubois *et al.*, (1956). Briefly, 0.5ml of the isolated and purified yogurt EPS (see section 3.2.12.1) was added into a test tube containing 0.5 ml of 5% phenol in 0.1 M hydrochloric

acid. The phenol-sulphuric acid reaction was activated by adding drop-by-drop concentrated sulphuric acid (2.5ml) into the test tube. The mixture was then vigorously vortexed prior to being left to cool to room temperature. Absorbance reading was taken at 490 nm against a reagent blank. All samples were run in triplicates. Glucose solutions (5-50 mg/ml) were used as a standard.

3.2.13 Sensory evaluation of yogurts

Sensory evaluation was conducted in the laboratory by 15 untrained panellists. Every sample was labelled using coded numbers and presented to the panellists. Panellists evaluated the samples by giving a score of 1-10 for the following criteria; body texture, aroma, visual appearance, sweetness, sourness and overall taste as described in Table 3.1. Panellists were instructed to rinse their mouth with the drinking water provided after evaluating each yogurt sample.

Table 3.1: Criteria evaluated in sensory evaluation.

| Criteria evaluated | Descriptions |
|----------------------|--|
| 1) Visual Appearance | Related to the attractiveness of the physical appearances and the colour of the yogurt. |
| 2) Aroma | Defined based on the sense of smell and could be referred to both pleasant and unpleasant odours that come out from the yogurt. |
| 3) Body Texture | Evaluated by pouring the yogurt onto the plates to see its viscosity and thickness. Texture was also evaluated by describing how the yogurt tastes in the mouth, whether it is thick, thin, greasy, or smooth. |
| 4) Taste | Evaluated by distinguishing the flavour of the yogurt, the sourness and sweetness of the yogurt and the overall taste of the yogurt. |

3.2.14 Statistical analysis

All data were analysed using Minitab14 software. Mean values were obtained by averaging independent measurements. Data were presented as mean \pm standard error mean. Difference between control and experimental groups were considered significant at $p < 0.05$.

4. RESULTS

4.1 DPPH inhibition: Preliminary experiment

Preliminary studies were carried out to determine the optimum concentration of DPPH reagents required to get a consistent reading of DPPH inhibition activity.

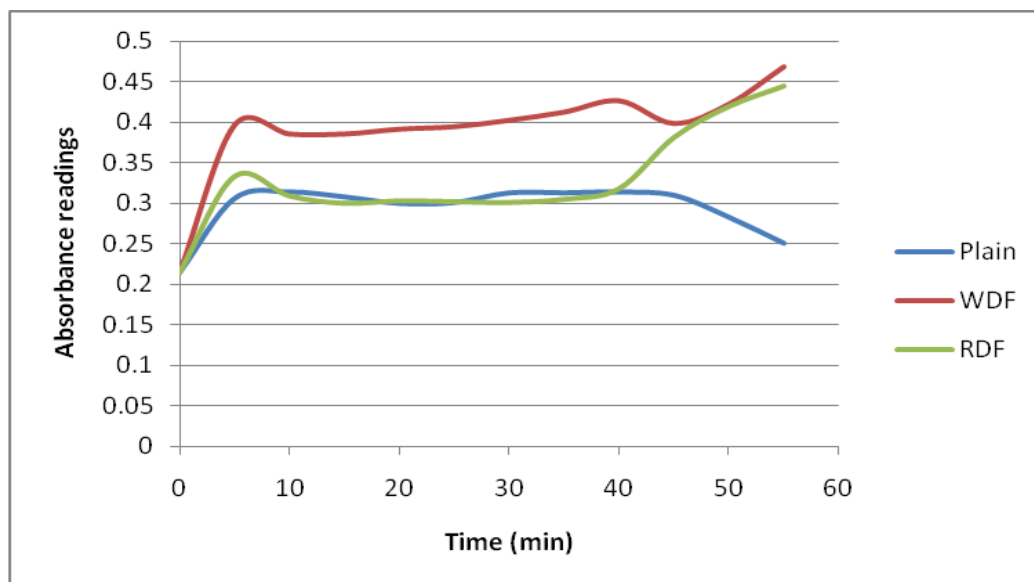


Fig. 4.1: The changes in absorbance readings at 517nm after the addition of yogurt water extracts in 30µM DPPH reagent.

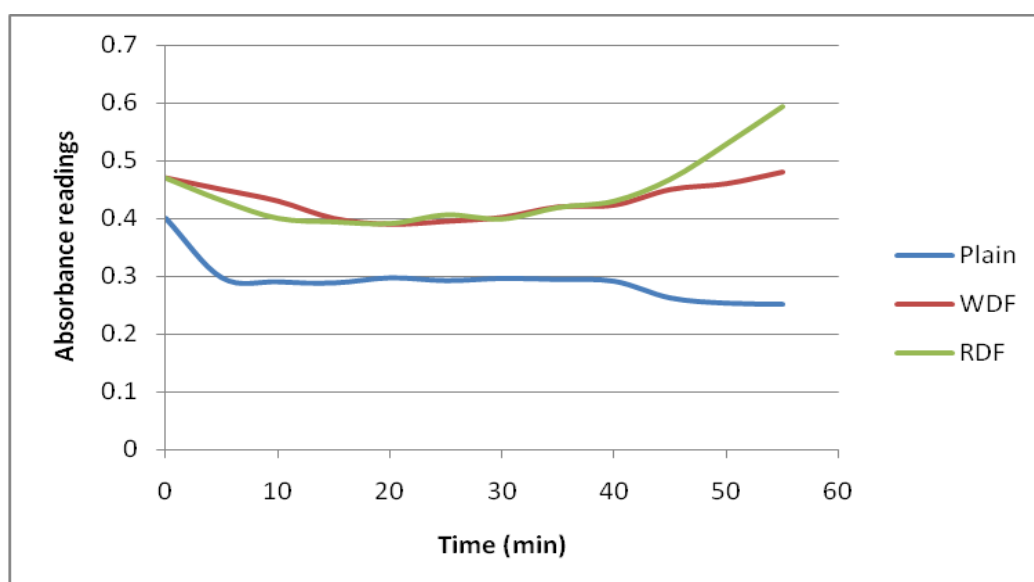


Fig. 4.2: The changes in absorbance readings at 517nm after the addition of yogurt water extracts in 60µM DPPH reagent.

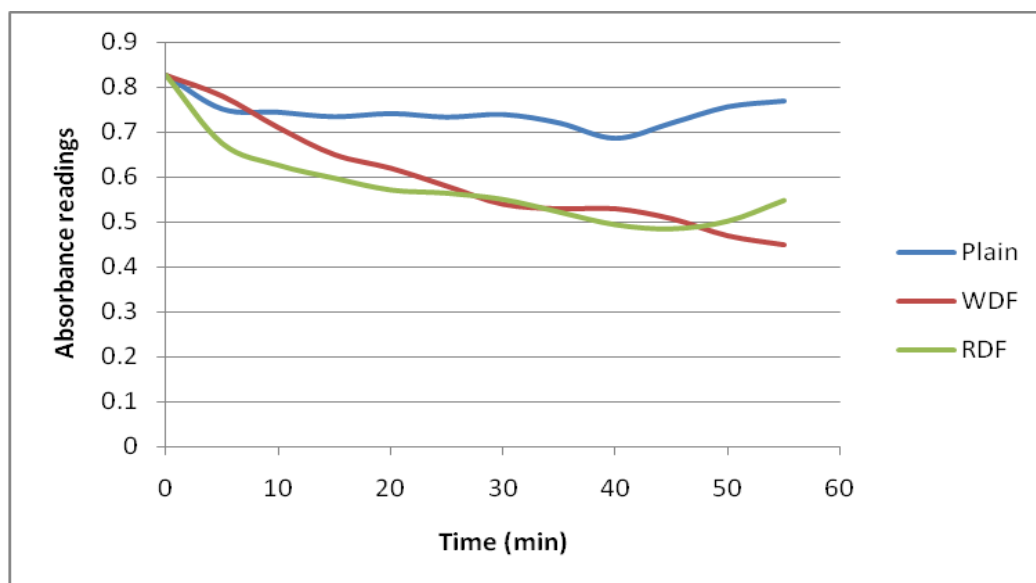


Fig. 4.3: The changes in absorbance readings at 517nm after the addition of yogurt water extracts in 120 μ M DPPH reagent.

The concentration of DPPH used influenced the antioxidant assay capacity (Fig. 4.1, 4.2, and 4.3). The use of 60 μ M of DPPH reagent as suggested by Shetty *et al.*, (2006) gave satisfactory inhibition effects which varied between samples. Higher concentration of DPPH reagent concentration (120 μ M) showed even higher inhibitory effects. However, the absorbance readings of white dragon fruit has not reached plateau by the 40th minute possibly due to the high of concentration of substrates present. Hence, 60 μ M of DPPH reagent concentration was chosen to be the most suitable for our system.

4.2 OPA assay: Preliminary experiment

Preliminary studies were carried out to determine the optimum reaction time required to get a consistent reading in OPA assay. Figure 4.4 shows the changes in absorbance readings of reaction mixture after the addition of yogurt water extract to OPA reagent. Every sample used gave the highest reading at about the 2nd minute of reaction. Thus, this reaction time was chosen for OPA assay.

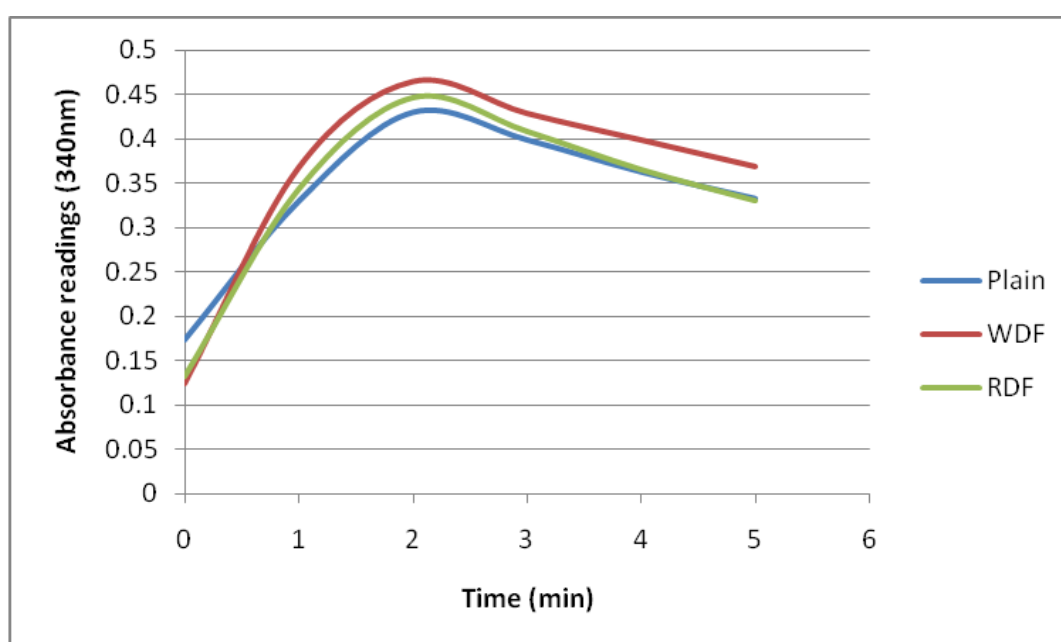


Fig. 4.4: The changes of absorbance readings with time after the mixing of yogurt water extracts with OPA reagent.

4.3 α -Amylase inhibition activity: Preliminary experiments

4.3.1 The optimum composition of DNSA reagent

Experiments were carried out to determine the best concentration of DNSA to be used in α -amylase inhibition assay. The optimal composition of a modified DNSA reagent was 2% (w/v) of DNS (Figure 4.5). The effects of varying the concentrations of the several components of the reagent on the absorbance reading in α -amylase inhibition assay were determined. In the absence of tartarate, the colour obtained with the modified reagent was unstable. To stabilize the colour under these conditions, 1ml of 18.2% tartarate solution was added to the mixture subsequent to the colour development and prior to cooling (Miller, 1959). Phenol and sodium bisulfite were eliminated from the modified reagent because the amount of reactants used in the assay were able to give rise to sufficient colour development without the need of further colour intensification. Furthermore, the sensitivity of the absorbance readings was decreased as the readings exceed 1.000 in the presence of phenol and bisulfite in the reagent (Figure 4.6).

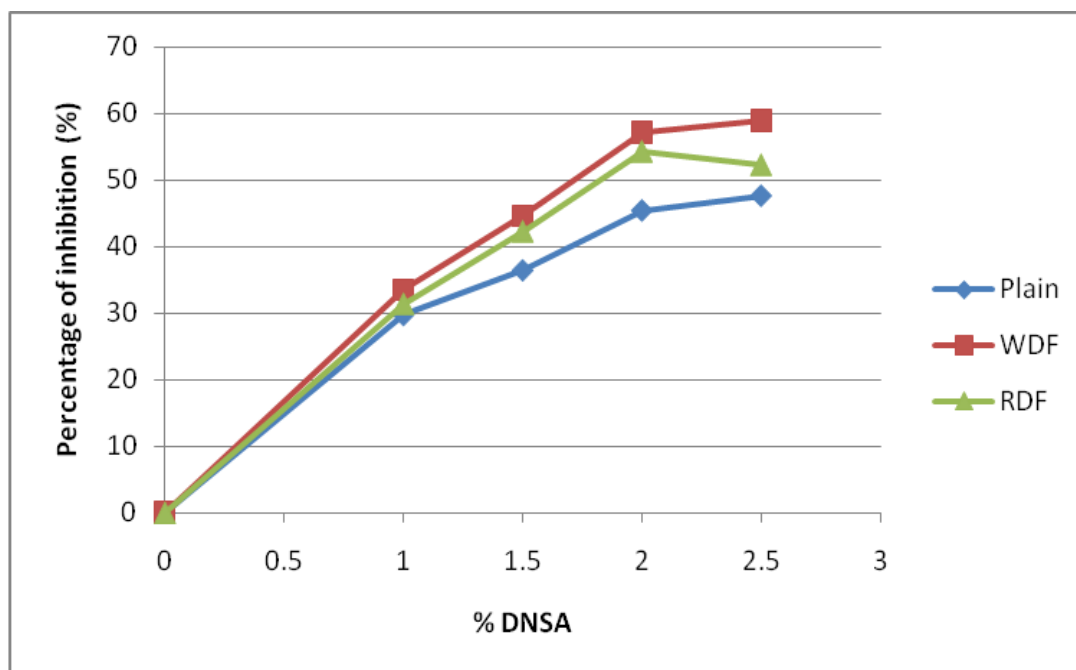


Fig. 4.5: The α -amylase inhibitory activity of yogurt water extracts at different concentration of DNSA.

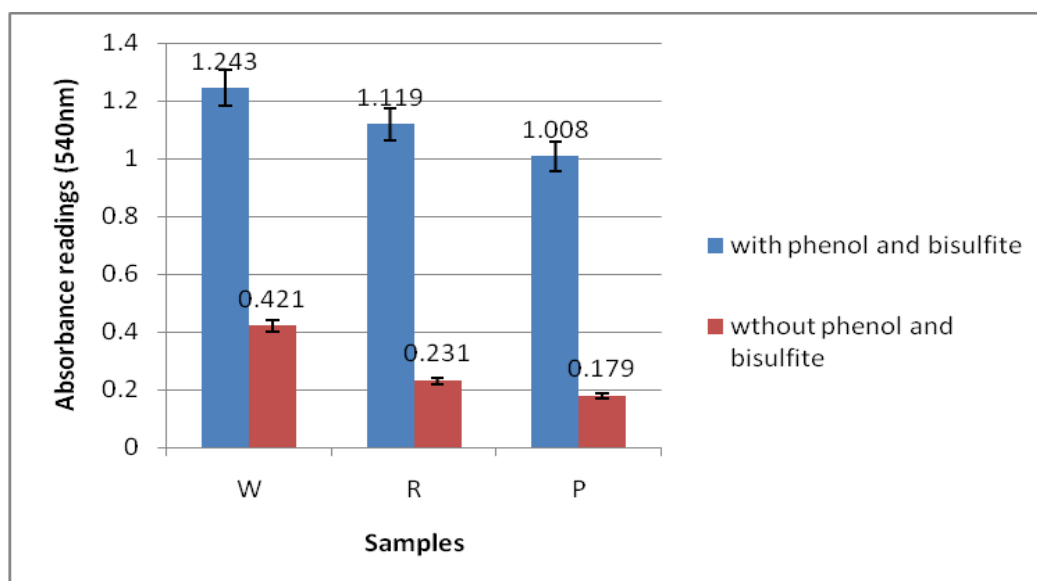


Fig. 4.6: Absorbance readings of yogurt water extracts at 540 nm with and without phenol and bisulfite in α -amylase inhibition assay

4.3.2 The optimum dilution factor of yogurt water extracts

Experiments were carried out to determine the best dilution factor of yogurt water extracts to be used in α -amylase inhibition assay. Undiluted yogurt water extract (neat; Figure 4.7) showed the highest inhibition and was chosen to determine the effect of *H.undatus* and *H.polyrhizus* yogurts on α -amylase inhibition.

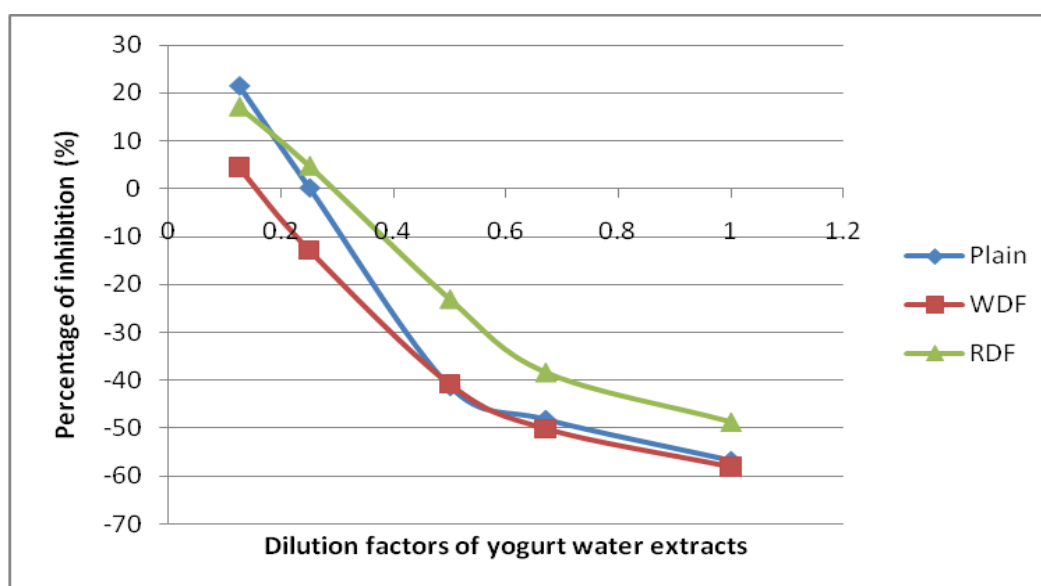


Fig. 4.7: The α -amylase inhibitory activity of yogurt water extracts at different dilution factors.

4.3.3 The optimum incubation time after the addition of substrate

Experiments were carried out to determine the optimum reaction time to enable α -amylase inhibition to be measured. The optimum incubation time for the inhibition of α -amylase after the mixing of enzyme, yogurt water extracts and starch (substrate) was chosen to be 10 minutes (Figure 4.8) because the inhibition was maximum at $t=10\text{min}$ prior to reaching plateau.

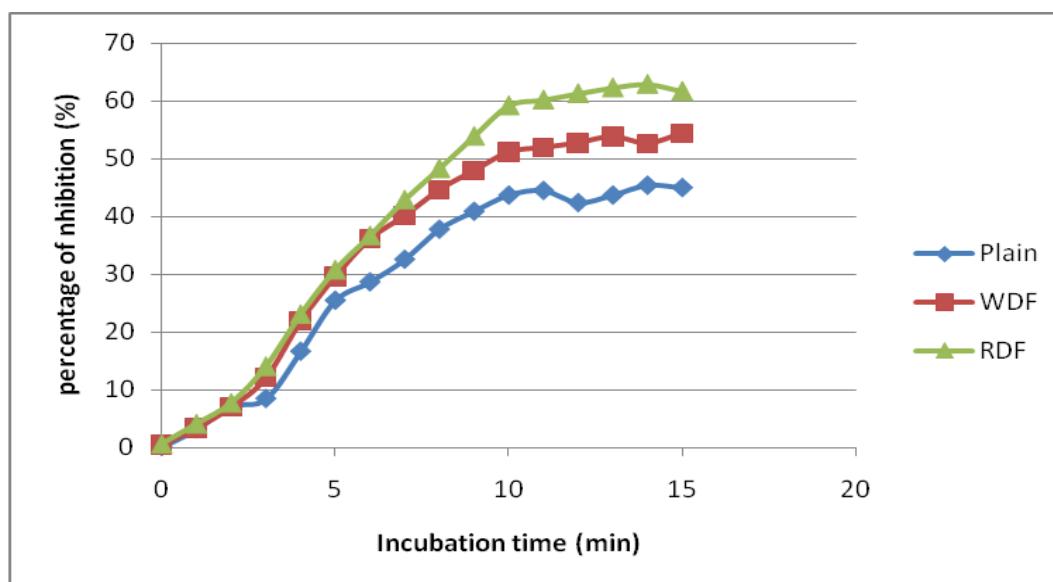


Fig. 4.8: The α -amylase inhibitory activity of yogurt extracts at different incubation time

4.3.4 The optimum boiling time after the addition of DNSA reagent

Preliminary studies were carried out to determine the best boiling time after the addition of DNSA reagent to be used in α -amylase inhibition assay. The optimum boiling time after the addition of DNSA reagent to the reaction mixtures was chosen to be 7 minutes (Fig.4.9). Longer boiling time of the reaction mixture leads to the saturation of the 3,4-dinitrosalicylic acid, as shown by the plateau state.

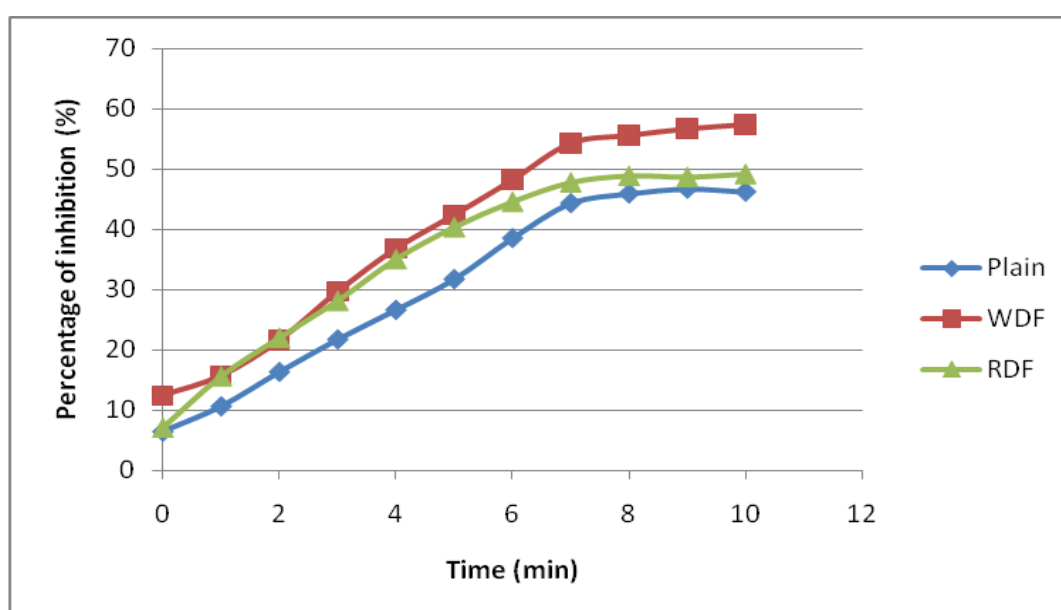


Fig. 4.9: The α -amylase inhibitory activity of yogurt water extracts at different boiling time.

4.4 α -Glucosidase inhibition assay: Preliminary experiments

4.4.1 The optimum enzyme concentration and incubation time

Experiments were carried out to determine the best enzyme concentration and reaction time to enable α -glucosidase inhibition assay to be measured. Figure 4.10 shows a hyperbolic curve was obtained for both neat (no dilution) and the 2 times diluted (2X) enzyme solution, whereas a linear curve was obtained for the 4 times diluted (4X) enzyme solution during the 25 minutes observation period. Besides the low yield of product (p-nitrophenol, in terms of absorbance readings), both 2X and 4X diluted enzyme solution were also shown to take relatively longer time to achieve equilibrium. Neat (undiluted) enzyme solution was chosen as it yields the maximum amount of product by the 10th minute before the reading (enzyme reaction) went plateau. Therefore, the optimum enzyme concentration and incubation time were neat (no dilution) and 10 minutes respectively.

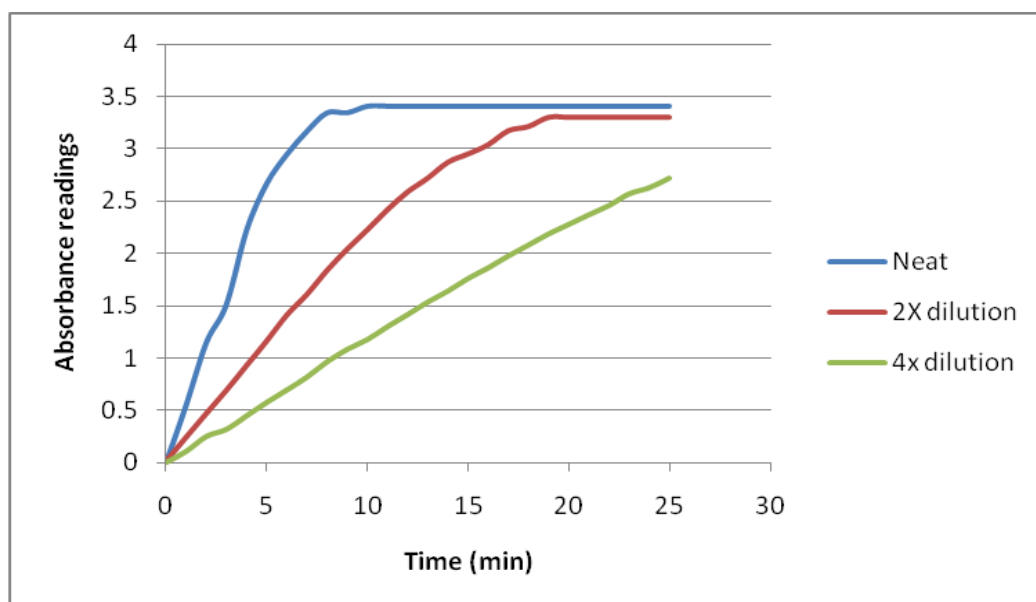


Fig. 4.10: The α -glucosidase activity of control sample (without inhibitor) with different dilution factors of enzyme solution.

4.4.2 The optimum dilution factor yogurt water extract

Preliminary studies were carried out to determine the best dilution factor to be used in α -glucosidase inhibition assay. Undiluted yogurt water extracts (neat) was chosen as they showed the highest α -glucosidase activity at the 10th minute (Figure 4.11). In the limiting frame of 25 minutes, a hyperbolic curve was obtained for both neat and the 2 times diluted (2X) yogurt water extracts, whereas, a linear curve was obtained for the 4 times diluted (4X) yogurt water extract.

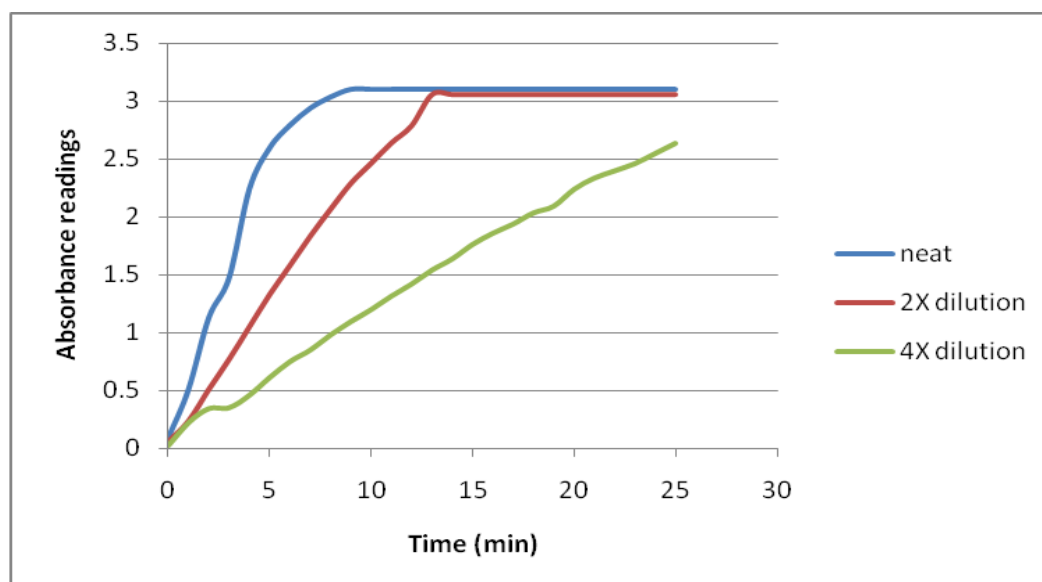


Fig. 4.11: The α -glucosidase activities of plain yogurt water extract at different dilution factors

4.5 Experiments on plain and dragon fruit-yogurt

4.5.1 Reduction of yogurt pH during fermentation

The presence of red dragon fruit enhanced the milk fermentation rate. White dragon fruit yogurt at 20% and 30% w/w showed increment ($p < 0.05$) in fermentation rate (-0.3471 and -0.3609 pH/h) compared to plain yogurt (-0.3369 pH/h; Figure 4.12). All dragon fruit yogurts showed significantly lower pH reading ($p < 0.05$) (pH 3.91-3.98) compared to plain (pH 4.38) yogurt by the 7.5 hour of fermentation. Red dragon fruit yogurts also showed faster rates of pH reduction (-0.3606 to -0.4126 pH/h) compared to plain yogurt (-0.3369pH/h).

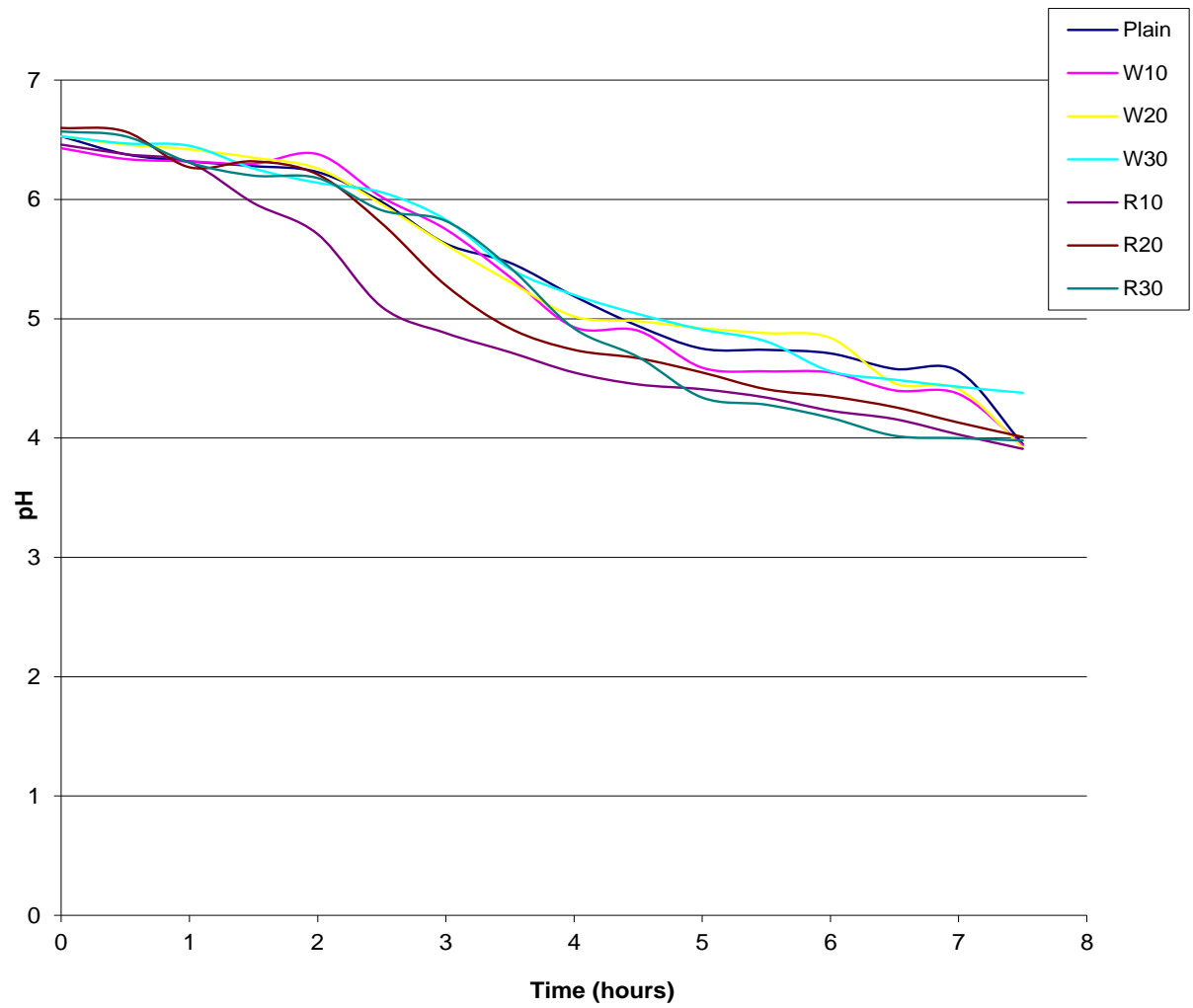


Fig. 4.12: Changes in pH during yogurt fermentation in the absence or presence of dragon fruit (10, 20 or 30% w/w).

The pH values of all yogurt samples on the 14th day of storage were decreased to 4.0 (Table 4.1). The pH of plain yogurt on the 14th day of refrigerated storage was 3.85 ± 0.03 . The addition of either white or red dragon fruits in increasing amount resulted in increased ($p < 0.05$) reduction of pH. White and red dragon fruits at 30% w/w showed the lowest pH (3.78 ± 0.02 and 3.75 ± 0.02 respectively; $p < 0.05$).

Table 4.1

pH values of plain and dragon fruit-yogurt on the 14th day of storage \

| Sample | pH |
|---|-----------------|
| Plain yogurt | 3.85 ± 0.03 |
| Yogurt + white dragon fruit extract (10% w/w) | 3.83 ± 0.04 |
| Yogurt + white dragon fruit extract (20 %w/w) | 3.81 ± 0.03 |
| Yogurt + white dragon fruit extract (30% w/w) | 3.78 ± 0.02 |
| Yogurt + red dragon fruit extract (10% w/w) | 3.81 ± 0.03 |
| Yogurt + red dragon fruit extract (20% w/w) | 3.79 ± 0.03 |
| Yogurt + red dragon fruit extract (30% w/w) | 3.75 ± 0.02 |

4.5.2 Titratable acid

Table 4.2: Titratable acid (TA) of dragon fruit yogurts on the 14th day of storage

| Sample | % |
|---|-----------------|
| Plain yogurt | 1.21 \pm 0.01 |
| Yogurt + white dragon fruit extract (10% w/w) | 1.34 \pm 0.01 |
| Yogurt + white dragon fruit extract (20 %w/w) | 1.39 \pm 0.02 |
| Yogurt + white dragon fruit extract (30% w/w) | 1.32 \pm 0.01 |
| Yogurt + red dragon fruit extract (10% w/w) | 1.37 \pm 0.01 |
| Yogurt + red dragon fruit extract (20% w/w) | 1.30 \pm 0.03 |
| Yogurt + red dragon fruit extract (30% w/w) | 1.28 \pm 0.01 |

All dragon fruit yogurts had higher titratable acid (Figure 4.13) after 7.5 hour fermentation (1.14 \pm 0.03 - 1.23 \pm 0.02 %) compared to plain yogurt (1.08 \pm 0.01%; $p < 0.05$). Yogurt with 20% w/w white dragon fruit showed the highest TA (1.23 \pm 0.02%) by the 7.5 hour fermentation. All dragon fruit yogurts had higher titratable acid (TA) on the 14th day of storage (1.18 \pm 0.01 - 1.29 \pm 0.02 %) ($p < 0.05$) compared to plain yogurt (1.11 \pm 0.01 %) (see Table 4.2). White dragon fruit yogurt at 20% w/w showed the highest TA (1.39 \pm 0.02%) on the 14th day of storage.

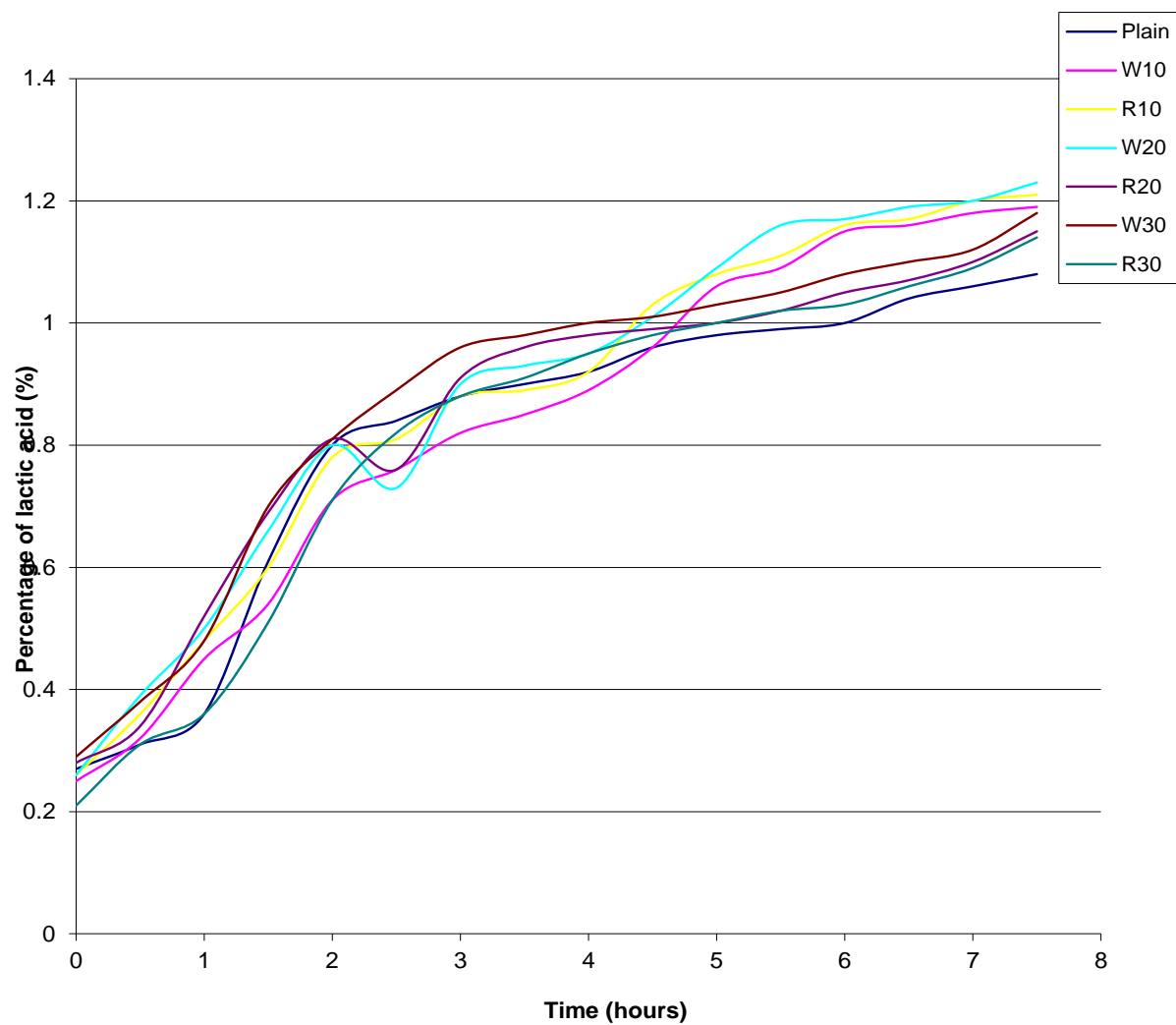


Fig. 4.13: Changes in titratable acid (lactic acid percentage) during fermentation of yogurt.

4.5.3 Syneresis measurement

The presence of dragon fruit resulted in higher syneresis (56.57 to 70.32%) compared to plain yogurt (52.93%; Fig.4.14). Highest syneresis was seen in 30 % w/w red dragon fruit yogurt (70.32%) followed by 30% w/w white dragon fruit yogurt (63.19%), 20% w/w red dragon fruit yogurt (61.24%), 10% w/w red dragon fruit yogurt (57.76%), 10% w/w white dragon fruit yogurt (57.19%) and 20% w/w white dragon fruit yogurt (56.57%) ($p < 0.05$).

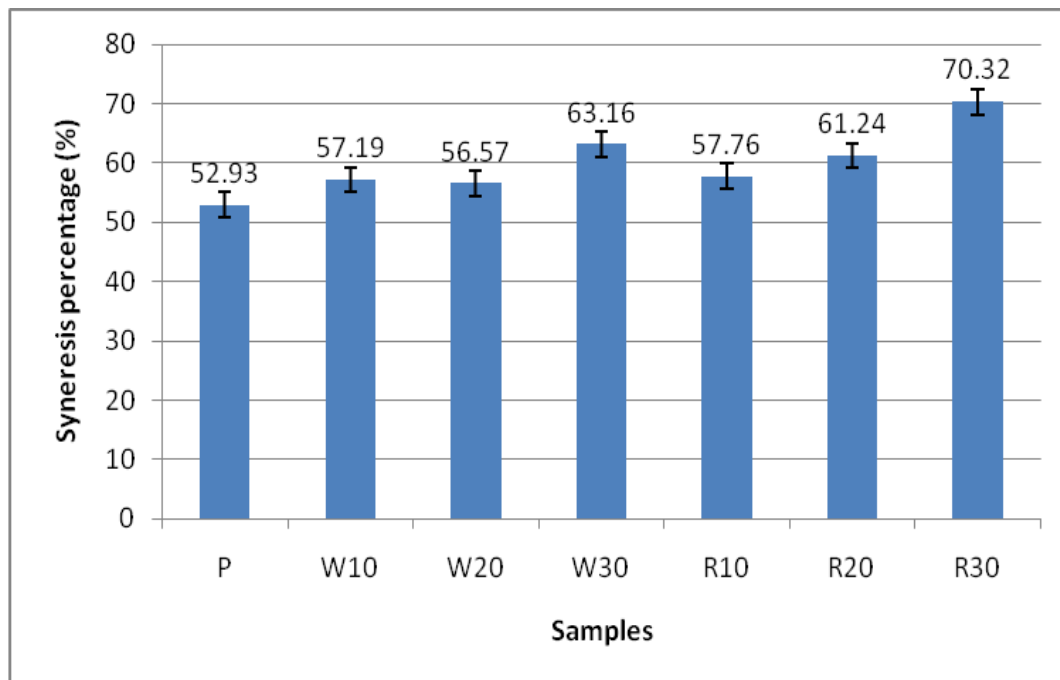


Fig. 4.14: Changes in syneresis in yogurt in the presence of dragon fruit. P (plain yogurt) W10, W20, W30 (yogurt containing white dragon fruit at 10, 20, 30% w/w respectively) R10, R20, R30 (yogurt containing red dragon fruit at 10, 20, 30% w/w respectively).

4.5.4 Total phenolic content (TPC) in dragon fruit yogurts

The addition of dragon fruit increased the TPC of yogurt (Fig.4.15). Yogurts containing white and red dragon fruit at 10% w/w inclusion showed similar yogurt TPC (i.e ~ 16ug/ml increase). However, increment of 20 and 30% of dragon fruit addition into yogurt did not result in similar graduated increase in TPC in white (54.34 and 64.43ugGAE/ml respectively) and red (43.22 and 49.61ugGAE/ml respectively) dragon fruit yogurts. Yogurt at 30 % (w/w) white dragon fruit inclusion showed the highest TPC (64.43ugGAE/ml) in comparison to 30% w/w red dragon fruit yogurt (49.61ugGAE/ml).

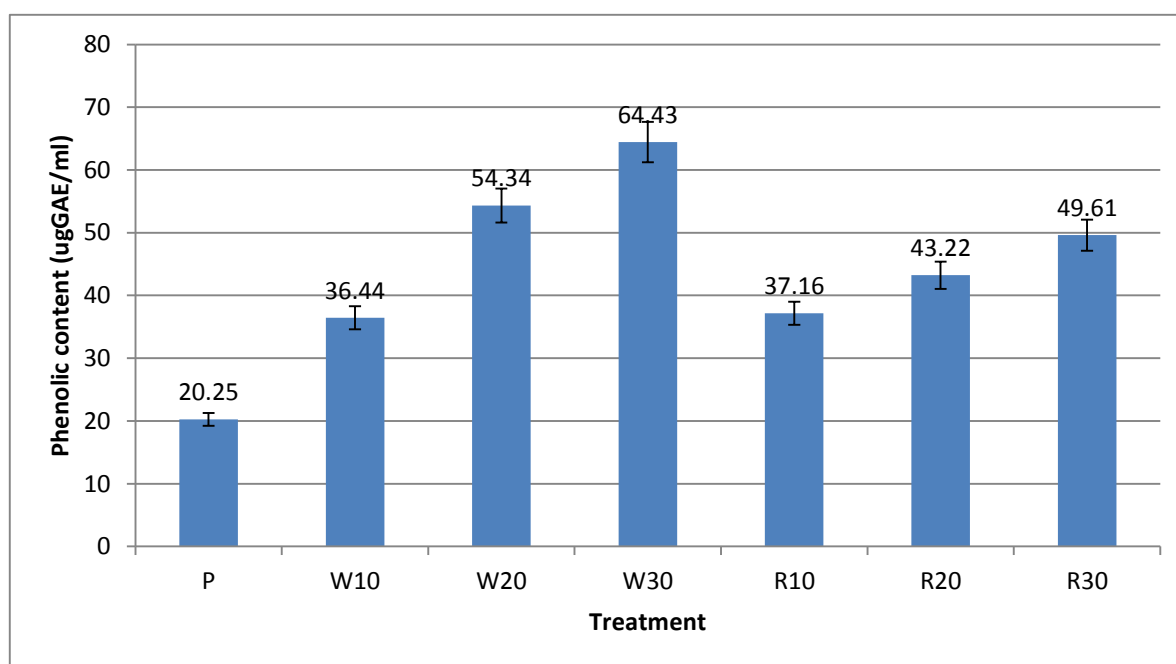


Fig. 4.15: Total phenolic content in red and white dragon fruit yogurts.

4.5.5 Antioxidant activity of dragon fruit-yogurts

Increasing inclusion of dragon fruit increased the antioxidant activities of yogurt ($p < 0.05$; Figure 4.16) compared to plain yogurt (19.16%). Red dragon fruit (30% w/w) showed the highest percentage of inhibition (45.74%) followed by red dragon fruit yogurt at 20% w/w inclusion (42.26%), white dragon fruit yogurt at 30% w/w inclusion (39.96%), red dragon fruit yogurt at 10% w/w inclusion (33.71%), white dragon fruit yogurt at 20% w/w inclusion (33.60%) and white dragon fruit yogurt at 10% w/w inclusion (24.97%).

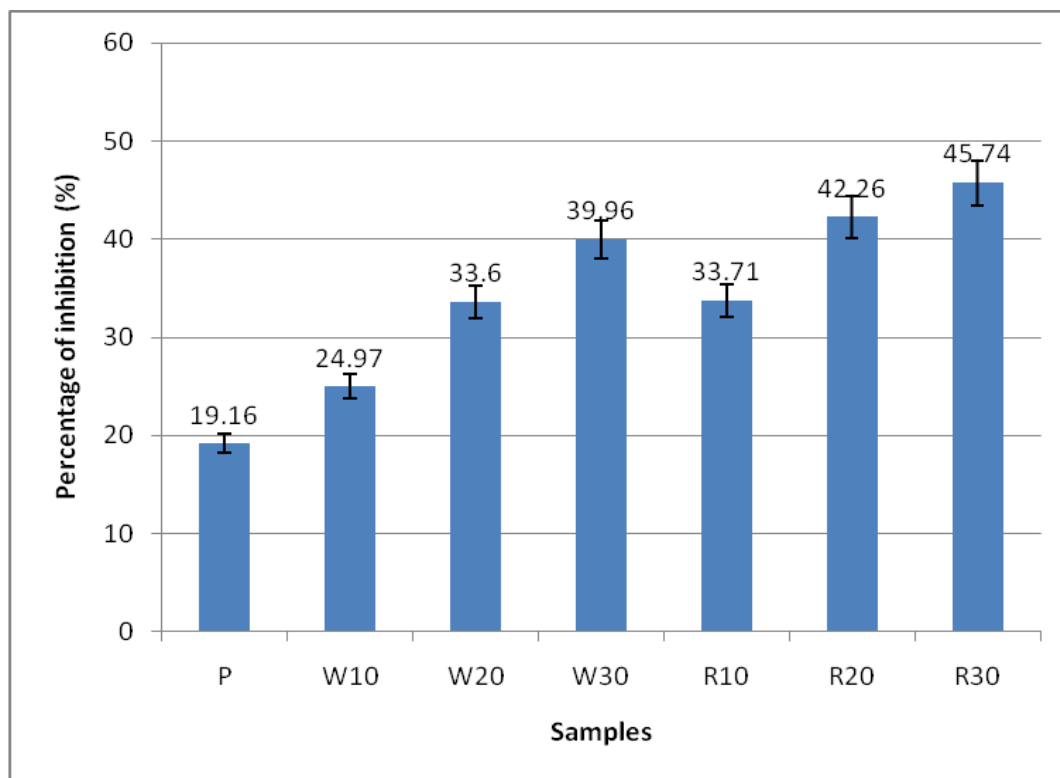


Fig. 4.16: DPPH antioxidant activity in yogurts. Values represent mean of 3 independent experiments ($n=3$) and bar represent standard error on the means (SEM).

4.5.6 Effects of dragon fruit extract on proteolysis of milk protein

Figure 4.17 shows O-phthaldehyde assay (OPA) values in fresh yogurts determined from regression equation of tryptone standards ($y=0.548x + 0.0$ $r^2=0.979$). The addition of dragon fruit did not increase yogurt proteolysis compared to plain yogurt ($p=0.061$). These results may also suggest that presence of *Hylocereus undatus* and *Hylocereus polyrhizus* in yogurt did not change the peptide content in yogurt compared of yogurt.

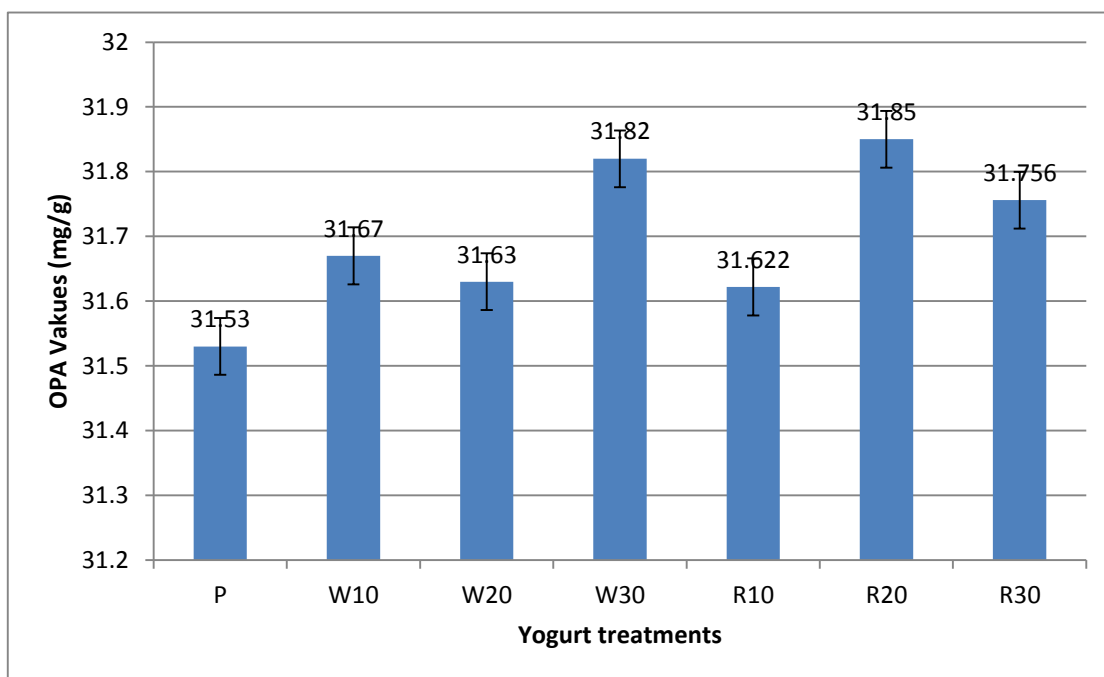


Fig. 4.17: Proteolysis in dragon fruit yogurts as determined using O-phthaldehyde assay.

4.5.7 α -Glucosidase inhibition assay

Yogurts containing dragon fruits except yogurt with 10% w/w of white dragon fruit had higher inhibitory potential ($p < 0.05$) compared to plain yogurt (9.21%; Figure 4.18). The highest inhibition of α -glucosidase was shown by white dragon fruit yogurt at 30% w/w inclusion (37.10%) followed by red dragon fruit yogurt at 30% w/w inclusion (34.02%), red dragon fruit yogurt at 20% w/w inclusion (27.36%), white dragon fruit yogurt at 20% w/w inclusion (19.79%) and red dragon fruit yogurt at 10% w/w inclusion (15.69%). Red dragon fruit (10%) showed higher inhibition (15.69%; $p < 0.05$) on α -glucosidase activity in comparison to plain (9.21%) and 10% w/w white dragon fruit yogurt (8.7%) yogurts. Increasing the incorporation dragon fruit into yogurts to 20% w/w resulted in similar increase in the inhibition of α -glucosidase activity for both red and white dragon fruit yogurts. Red dragon fruit yogurts (15.59%) showed more potent effect than white dragon fruit yogurts (8.7%) on α -glucosidase inhibition at 10% inclusion.

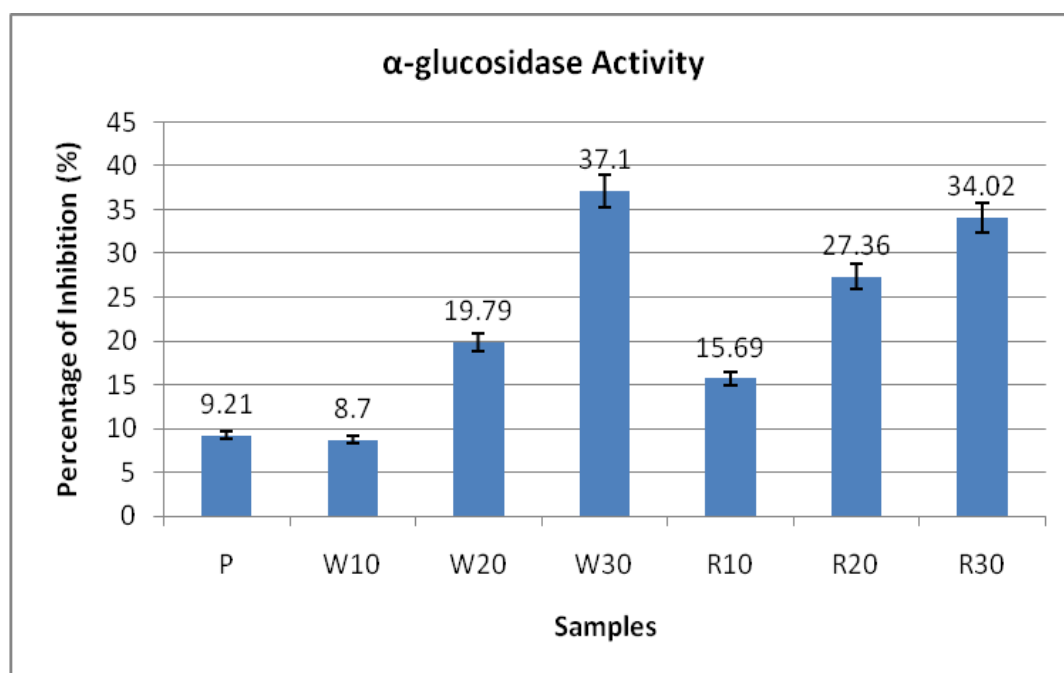


Fig. 4.18: Inhibition on α -glucosidase activity by dragon fruit yogurts extracts.

However, the increment of α -glucosidase inhibition in red dragon fruit yogurt was in a linear form while the increment of α -glucosidase inhibition in white dragon fruit yogurt was in an exponential form (Figure 4.19). This is reflected in the smaller IC_{50} values by 10% w/w red dragon fruit yogurt (82.5mg) than that by 10% w/v white dragon fruit yogurt (147.98mg; $p < 0.05$). Higher addition (20% w/w) of dragon fruit into yogurt resulted in further lowering of IC_{50} value by red dragon fruit yogurt (48.1mg) compared to that by white dragon fruit yogurt (69.3mg). No difference in IC_{50} values was shown by yogurts at 30% w/w inclusion for both red and white dragon fruits.

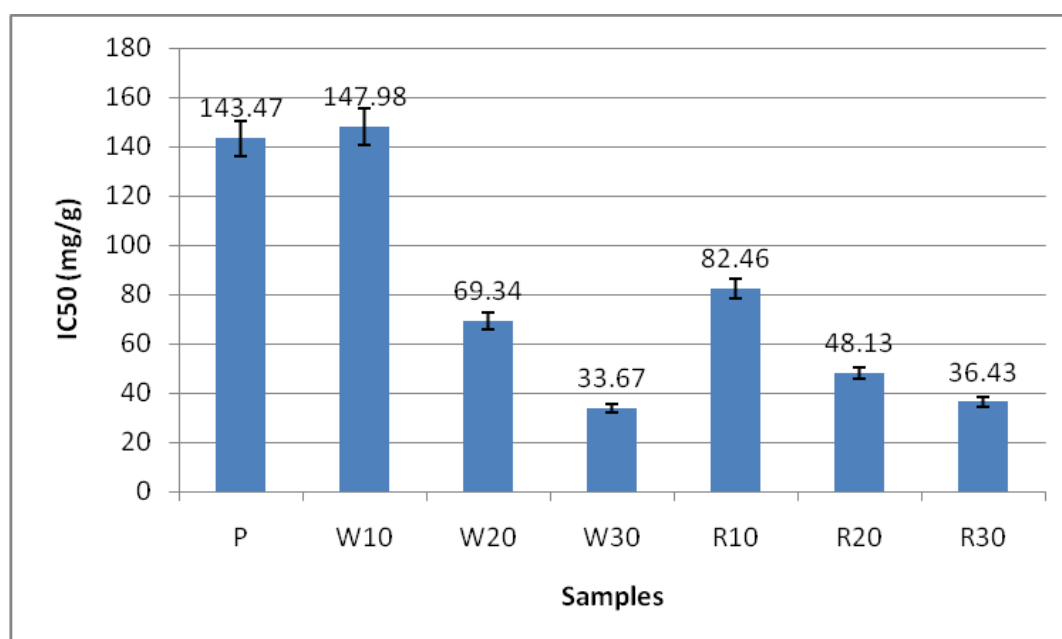


Fig. 4.19: IC_{50} values for α -glucosidase inhibition by dragon fruit yogurts.

4.5.8 α -Amylase inhibition assay

Dragon fruit yogurts except yogurt with 10% w/w of white dragon fruit had higher inhibitory potential on α -amylase compared to plain yogurt (19.71%; Figure 4.20). Red dragon fruit yogurt at 30% w/w inclusion showed the highest inhibition (52.2%) followed by red dragon fruit yogurt at 30% w/w inclusion (44.65%), red dragon fruit yogurt at 20% w/w inclusion (38.40%), white dragon fruit yogurt at 20% w/w inclusion (27.10%) and red dragon fruit yogurt at 10% w/w inclusion (22.20%). Red dragon fruit (10%) showed higher inhibition (22.20%; $p < 0.05$) on α -amylase activity in comparison to plain (19.71%). White dragon fruit yogurt at 10% w/w inclusion was not different from control (17.3%) yogurts.

Increasing the incorporation of dragon fruit into yogurts to 20% w/w resulted in similar increase in the inhibition of α -amylase activity for both red and white dragon fruit yogurts. Yogurt at 30% w/w of red dragon fruit inclusion (52.02%) showed less increase ($p < 0.05$) in inhibition of α -amylase activity compared to that by white dragon fruit (44.65%). Red dragon fruit yogurts (22.2%) showed more potent effect than white dragon fruit yogurts (17.3%; $p < 0.05$) on α -amylase inhibition at 10% inclusion. The IC_{50} of 10% w/v red dragon fruit yogurt (62.3mg/g) was significantly lower than that in 10% w/v white dragon fruit yogurt (74.37mg/g; $p < 0.05$; Figure 4.21). Higher addition (20% w/w) dragon fruit into yogurt resulted in lower IC_{50} value by red dragon fruit yogurt (33.2mg/g) compared to that by white dragon fruit yogurt (48.92mg/g). No difference in IC_{50} values was shown by yogurts at 30% w/w inclusion of red and white dragon fruits.

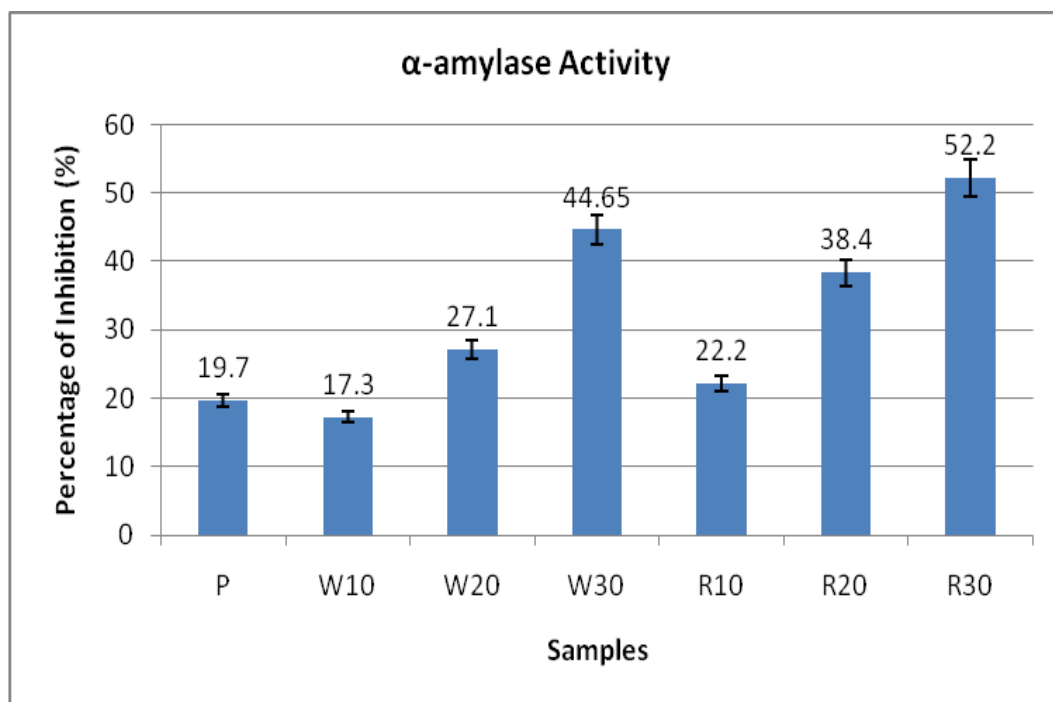


Fig. 4.20: Inhibition on α -amylase activity by dragon fruit yogurts extracts.

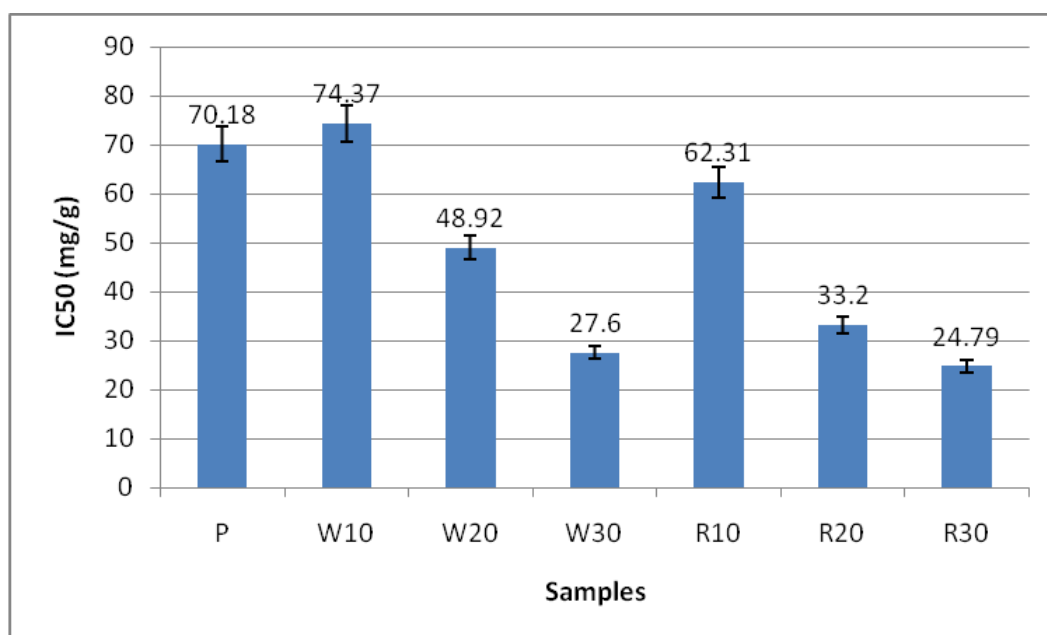


Fig. 4.21: IC₅₀ values for α -amylase inhibition by dragon fruit yogurts.

4.5.9 Sensory evaluation of yogurts

Sensory evaluation of dragon fruit yogurts (Figure 4.22) were carried out based on 6 sensory criteria i.e. visual appearance (VA), body texture (BT), aroma (A), sweetness (SW), sourness (SO), and overall taste (OT). Red dragon fruit yogurt showed the highest score for visual appearance (7.77), aroma (5.9) and sweetness (4.22) whereas plain yogurt gave the highest score for body texture (6.81), sourness (7.13) and overall taste (5.45). Both red and white dragon fruit yogurts in comparison to plain yogurt showed a significant differences ($p < 0.05$) in terms of visual appearance (7.77 & 5.81 vs 6.9), body texture (5.9 & 5.95 vs 6.81), sweetness (4.22 & 3.91 vs 3.43), and sourness (5.77 & 6.09 vs 7.13). No significant differences with respect to aroma and overall taste criteria ($p > 0.05$) between dragon fruit yogurts and plain yogurt.

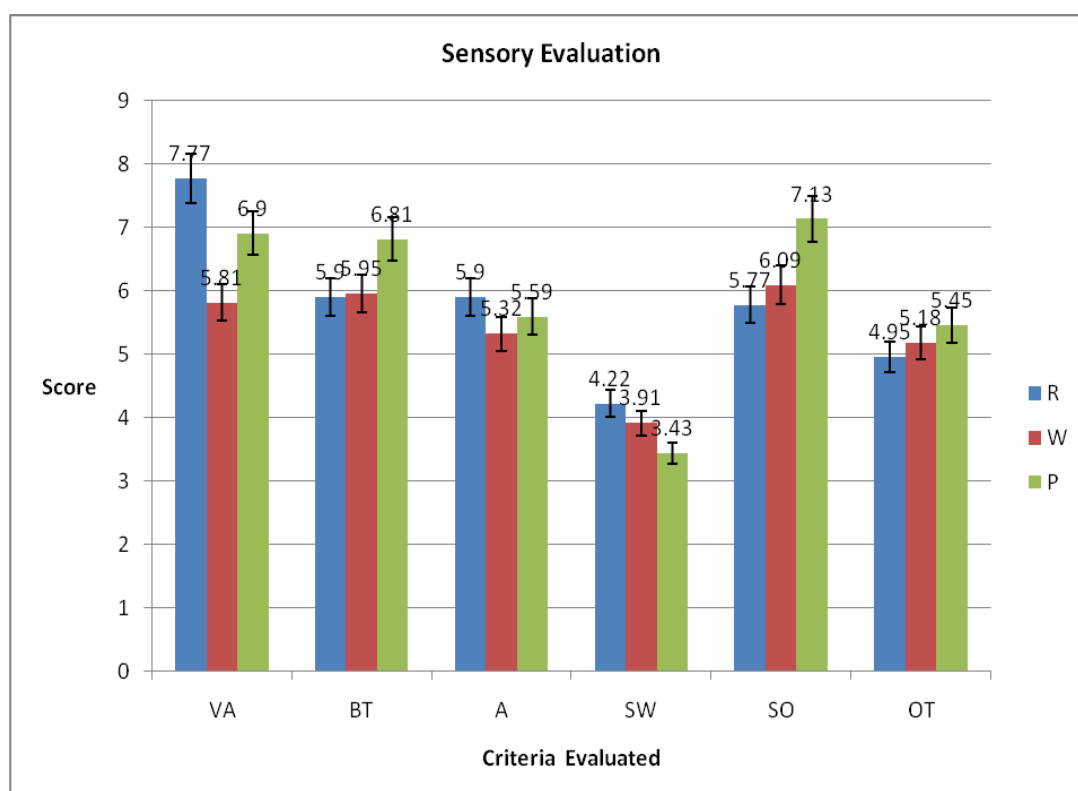


Fig. 4.22: Sensory evaluation score for plain and dragon fruit yogurts. P=Plain yogurt
W= White dragon fruit yogurt, R= Red dragon fruit yogurt.

4.5.10 Exopolysaccharides production of yogurts

All dragon fruit yogurts showed increased exopolysaccharides (EPS) content (214 - 738mg/L; $p < 0.05$) compared to plain yogurt (181mg/L; Fig. 4.23). Red dragon fruit yogurts at 20 and 30% w/w (549 and 738mg/L respectively) contained higher EPS than white dragon fruit yogurts (474 and 713mg/L respectively) for the same amount of fruit added. Yogurt at 30 % w/w red dragon fruit inclusion showed the highest EPS content (738mg/l) followed by yogurt at 30% w/w white dragon fruit inclusion (713mg/L), yogurt at 20% w/w red dragon fruit inclusion (549mg/L), yogurt at 20% w/w white dragon fruit inclusion (474mg/L) yogurt at 10% w/w white dragon fruit inclusion 288mg/l) and yogurt at 10% w/w red dragon fruit inclusion (214mg/L). The increase in EPS content with increasing dragon fruit inclusion occurred in a dose-dependent manner. Significant differences ($p = 0.036$) in EPS content were noted between white and red dragon fruit yogurts at 10% and 20% w/w but not at 30% w/w fruit inclusion ($p = 0.078$).

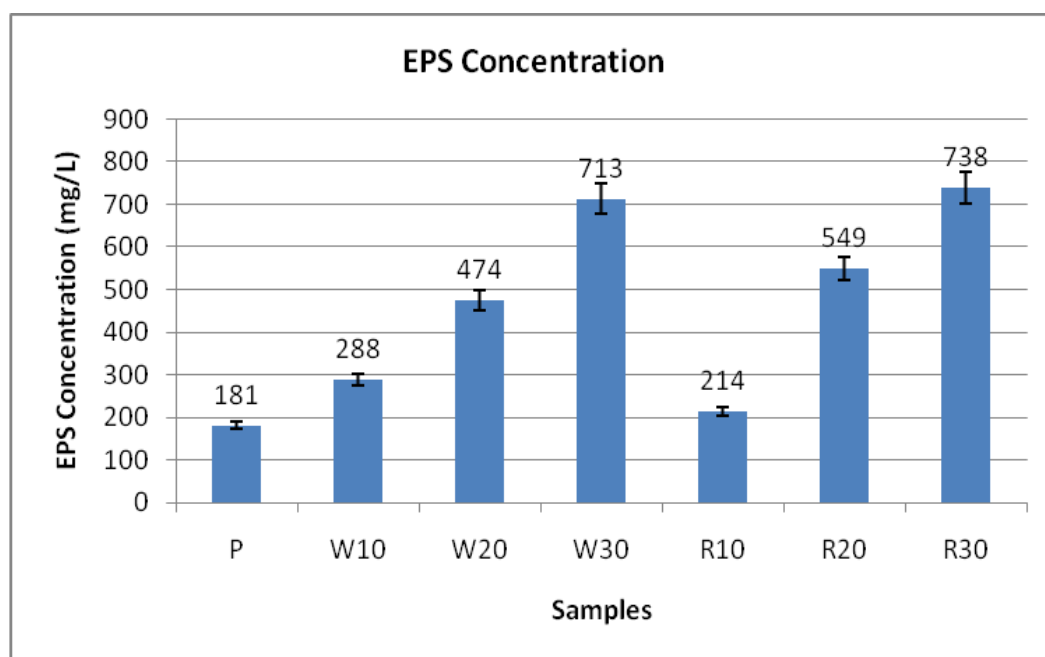


Fig. 4.23: Exopolysaccharides concentration of plain and dragon fruit yogurts.

5. DISCUSSION

5.1 Effects of dragon fruits on the changes of physicochemical properties of yogurt

5.1.1 pH of yogurt

The yogurt bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii*) used in the present studies grow optimally under anaerobic condition between 37-41°C. These lactic acid bacteria (LAB) utilize carbon sources as precursors to obtain energy with the formation of various organic acids (lactic acid, acetaldehyde, diacetyl etc) as metabolic by-products (Novak and Lubiere, 2000) which can be measured by pH and titratable acid. Both measurements are important because the former measures the H^+ whereas the later measures total organic acids present in yogurt. The differences in the rate of H^+ produced (pH reduction) may be used as an indicator for different growth rates of LAB in the yogurt (Adolfsson *et al.*, 2004).

In the present studies yogurts containing red or white dragon fruit had similar pH reduction patterns during fermentation (Figures 4.12). The initial pH values of white and red dragon fruits (10%, 20%, 30%)-yogurts (6.43 - 6.57) did not differ much from plain-yogurt (6.53), indicating the acidic content of the fruit extracts on yogurt pH were minimal. However, other studies showed changes in initial pH of yogurt (Kailasapathy *et al.*, 2007) in the presence of fruits. The declining of pH during fermentation was due to the protocoooperative action of two strains of yogurt bacteria i.e *S.thermophilus* and *L.bulgaricus* (Brabandere *et al.*, 1999). The extent of accumulation of all these fermentation products (lactic acid, acetaldehyde, diacetyl etc) reflects the high metabolic activity of the lactic acid bacteria.

LAB grow optimally under anaerobic condition between 37-41°C. However, these bacteria may be active even at refrigerated temperature resulting in noticeable pH

decrease during storage (Shah *et al.*, 1995). The presence milk sugar (carbon source) and milk protein (nitrogen source) in the rich medium of milk and optimum incubation temperature (41°C) encourage yogurt bacteria to grow rapidly (Savaiano *et al.*, 1987). Post acidification is the residual acid production after fermentation (Shah *et al.*, 1995), may be attributed to bacterial β -galactosidase, which is known to remain active at low storage temperature (0-5°C) (Marshall and Tamime, 1997). Therefore, it is suggested that the addition of white and red dragon fruits enhanced the milk fermentation rate and decrease the yogurt pH after 14 days of storage.

5.1.2 Titratable acid of yogurt

In the present studies, the decrease in pH during fermentation was associated with an increase in TA (Figures 4.12 and 4.13). The increment in TA by the 14th day of storage (Table 4.2) compared to that during fermentation (Fig. 4.13) can be attributed to post acidification activity in yogurt lactic acid bacteria (Tamime and Robinson, 1999; Oliveira *et al.*, 2009). TA are organic acids that are metabolically produced by the LAB during fermentation, and these may include lactic acid, acetic acid, propionic acid, citric acid, pyruvic acid, butyric acid and succinic acid (Ostlie *et al.*, 2003). A difference in TA production during fermentation was shown to be attributed to the differential microbial population (Prejapati and Dave, 1994). Increase in TA in the presence of fruits (Borges *et al.*, 2011; Gonzalez *et al.*, 2011) or plant extracts (Mocanu *et al.*, 2010; Michael, 2010; Shaaban *et al.*, 2010) have also been previously reported.

It would be interesting to know what proportions of acids are produced under different fermentation conditions attributed to the dragon fruits. An increase in TA may be associated with increased microbial metabolic activity under stress condition (Leroy *et al.*,

2003). In the present studies, the presence of dragon fruits could be viewed as creating favourable environmental condition for the LAB because both red and white dragon fruits contain high sugar content (Ming *et al.*, 1997) which can be utilized by them as carbon sources. Thus, it is suggested that the inclusion of white and red dragon fruit enhance the titratable acid production in yogurt.

5.1.3 Syneresis of dragon fruits yogurts

Syneresis is calculated as the percent weight of the separated whey over the initial weight of the gel. The addition of dragon fruit into yogurt increased syneresis (Figure 4.14). Common reasons for increased syneresis include exposure to high incubation temperature, excessive whey protein to casein ratio, low solids content and physical mishandling of the product during storage and retail distribution (Lucey, 2004). On the other hand, reduction of syneresis may be caused by the effect of transglutaminase on the pore size of the milk gels. As pore size reduces, the protein network will result in smaller syneresis (Lorenzen *et al.*, 2002).

The exact causes of whey separation in yogurt are not known. Although these methods give results with high precision, they do not represent the actual value of spontaneous syneresis in a set yogurt (Amatayakul, 2005). Breakage of the yogurt gel (Amatayakul *et al.*, 2006; Guache *et al.*, 2009; Lee and Lucey, 2010; Patel, 2011) as well as the presence of EPS (Amatayakul *et al.*, 2006; Ramchandran and Shah 2009; Zhang, 2012) may influence the result. The small difference ($p>0.05$) in syneresis between red dragon fruit yogurt and white dragon fruit yogurt suggest that may be syneresis is not only affected by physical factors.

Commercial preparation of yogurts attempt to minimise syneresis by increasing total solid content (14 to 16%) or by adding stabilizers like pectin and gelatin (Lucey *et al.*, 1998). The practice of using exopolysaccharides (EPS)-producing stater cultures in yogurt making showed considerable success to minimize syneresis (Amatayakul *et al.*, 2006). But in the present studies, the addition of white and dragon fruit which increased EPS production (See section 5.4) was not able to help to reduce syneresis in yogurt.

5.2 Effects of dragon fruits on therapeutical properties of yogurt

5.2.1 Total phenolic content (TPC)

TPC measurement is very important in the evaluation of antioxidant contents in food. The TPC values only give a general measure of phenolic content because the Folin-Ciocalteu method determines total phenols (and other easily oxidized substances) and it is not specific for particular phenolic compounds. Yogurts containing various concentrations of dragon fruits showed significantly higher TPC compared to plain yogurt (Figure 4.15). The TPC content in dragon fruits is comparable to jackfruit, pineapple, papaya, pomelo, and yellow pear and is even higher than honey dew, red watermelon and sugar cane (Isabelle *et al.*, 2010) Thus, white and red dragon fruit can be a good dietary source of phenolic compounds when added to yogurt. Higher TPC was reported by McCue *et al.*, (2005) and Shetty *et al.*, (2006) for a variety of dairy and soy yogurt (40-450ugGAE/ml) compared to the results from the present studies (20.2-64.3ugGAE/ml).

The TPC value in plain yogurt partially represent milk protein breakdown products (Oliveira *et al.*, 2009) and the content of amino acid such as tyrosin which has a phenolic side chain which may increase the TPC value in plain yogurt (Shah 2000). Other possibilities are microbial utilization of phenolic acids such as ferulic and p-coumaric acid during fermentation process and post acidification may lead to the production of other

phenolic acids such as vanillic and p-hydroxybenzoic acids before the aromatic ring structure is broken down (Blum, 1998).

Many phenolic compounds can play a major role in the protection of oxidation processes because they are able to scavenge reactive oxygen species due to their electron donating properties (Velioglu *et al.*, 1998). Their antioxidant effectiveness in food depends on not only the number and location of hydroxyl groups but also on factors such as physical location, interaction with other food components, and environmental conditions (e.g., pH) (Pitchaon, 2006). In many studies, phenolic compounds demonstrated higher antioxidant activity than antioxidant vitamins and carotenoids (Re *et al.*, 1999; Velioglu *et al.*, 1998).

5.2.2 Antioxidant activity by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical inhibition assay

The DPPH method is widely applied for the measurement of antioxidant activity of polyphenolics and colourants (Cai *et al.*, 2003; Shi & Le Maguer, 2000) because it exclusively determines the intrinsic ability of a sample to donate hydrogen atoms or electrons to this reactive species in a homogeneous system (Wang *et al.*, 2006). In the present studies, both dragon fruit yogurts showed higher antioxidant capacity compared to plain yogurt (Figure 4.17). Red dragon fruit inclusion at 10% produced yogurt with more antioxidant activities (35.7%) than that caused by white dragon fruit (24.9%). This could be explained by the higher vitamin C in red than in white dragon fruit yogurt (Isabelle *et al.*, 2010) and higher TPC in white than in red dragon fruit (Figure 4.16). Increment of another 10 and 20% of dragon fruit produced relatively more antioxidant activities for white dragon fruit yogurt (33.6 and 39.9% respectively) than for red dragon fruit yogurt (42.3 and 45.7% respectively).

Weak ($r^2=0.227$) and moderate ($r^2=0.493$) correlation was found between the phenolic content and antioxidant capacity for dragon fruit yogurts and plain yogurt respectively possibly because of the content of phenolic compounds and vitamin C in both

dragon fruits which are known to have antioxidant activity (Proteggente *et al.*, 2002). A study by McCue *et al.*, (2004) showed higher antioxidant activities for soymilk and soy yogurt (85.8-92.3%) compared to the results from the present studies (19.1-45.7%). This could be related to the fact that soya bean has higher TPC (McCue *et al.*, 2005; Shetty *et al.*, 2006) and vitamin C (Kumar *et al.*, 2010; Valente *et al.*, 2011) compared to dragon fruits. The amount of dragon fruits added into the yogurt could also relate to the antioxidant activities. Studies using *euterpe* juice for instance showed lower inhibition percentage for plain yogurt, commercial fruit enriched yogurt, and yogurt with *euterpe* juice (9.97-32.66%; Caisson *et al.*, 2005) compared to the present studies.

Red dragon fruit has recently drawn much attention of growers worldwide, not only because of their red-purple colour and economic value as food products, but also for their antioxidative activity from the betacyanin contents (Wybraniec & Mizrahi, 2002). The most important red dragon fruit pigments are the betacyanins and betaxanthins (Wybraniec *et al.*, 2001). Phenolic compounds influence antioxidant-activity measurement. They interfere with the oxidation process by reacting with free radicals, chelating catalytic metals, and scavenging oxygen. The effect of dragon fruit addition on antioxidant activity could be a result of the types of polyphenolics they contained. An increase in the number of hydroxyl groups (–OH) or other hydrogen-donating groups ($\equiv\text{NH}$, –SH) in the molecular structure led to higher antioxidant activity (Cai *et al.*, 2003). Betanins contain amino groups and hydroxyl groups and would contribute to antioxidant activity of dragon fruit flesh (Cai *et al.*, 2003), which could partly explain yogurt containing dragon fruit extracts is a better antioxidant than plain yogurt.

5.2.3 α -Glucosidase inhibitory potentials of yogurt

Diabetes mellitus is an emerging health concern globally, and is characterized by hyperglycemia, a condition in which blood glucose rise to abnormal level (Apostolidis *et al.*, 2006). Ability to control blood glucose is important to diminish the risk of type-2 diabetes and the inhibition of intestinal α -glucosidase, an enzyme that responsible in the digestion of disaccharides to absorbable monosaccharides (Palanuvej *et al.*, 2009), is a practical intervention approach. α -Glucosidase is an intestinal enzyme which catalyzes the degradation of dietary polysaccharides to absorbable monosaccharides.

All dragon fruit yogurts showed higher ($p<0.05$) inhibition on α -glucosidase activity compared to plain yogurt (Figure 4.18 and 4.19). In particular, white dragon fruit yogurts showed more increment on α -glucosidase activity inhibition ($p<0.05$) compared to red dragon fruit yogurts. These suggest phytochemicals in white dragon fruit are different from red dragon fruit. Inhibition of α -glucosidase may be explained by the changes in protein structures (McCue and Shetty, 2004) and total phenolic content (Shetty *et al.*, 2006; Kwon *et al.*, 2010) because phenolics are known to bind to the reactive sites of enzymes thus altering its catalytic activity (McCue & Shetty, 2004).

There was relatively weak correlation between α -glucosidase inhibition and the peptide content in white dragon fruit yogurt, red dragon fruit yogurt and plain yogurt ($r^2=0.134$, 0.216 , and 0.257 respectively). No correlation was found between α -glucosidase inhibition and the total phenolic content in white dragon fruit yogurt, red dragon fruit yogurt and plain yogurt ($r^2=0.057$, 0.077 , and 0.043 respectively). The poor correlation may be explained by the lower α -glucosidase inhibition in the present studies (9-34%) compared to Shetty *et al.*, (2006) which reported higher α -glucosidase inhibition for a variety of dairy and soy yogurts (30-70%).

5.2.4 α -Amylase inhibitory potentials of yogurt

The inhibition pancreatic α -amylase was also seen in the presence of *H. undatus* and *H. polyrhizus*. The inhibition of this rate-limiting enzyme in carbohydrate metabolism could serve as an approach to suppress rapid increase in blood glucose, a condition commonly found in type-2 diabetes (Shetty *et al.*, 2006). Inhibitors of these enzyme delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently suppressing the postprandial plasma glucose rise (Wild *et al.*, 2004). This can be a promising strategy in the management of type-II diabetes (Shetty *et al.*, 2006). Research on the development and utilization of natural food grade anti-diabetic plant ingredients with optimal inhibition and without side effects are being investigated. The objective of the present study was to show the potential antidiabetic effects of phenolics by inhibition of carbohydrate hydrolyzing enzymes such as α -amylase and α -glucosidase.

All dragon fruit yogurts showed higher ($p < 0.05$) inhibition on α -amylase activity except for yogurt with 10% inclusion of white dragon fruit (Figure 4.20 and 4.21). A study by Shetty *et al* (2006) showed α -amylase inhibition (5-45%) for a variety of dairy and soy yogurts which are comparable the present studies (19-52%). In particular, total phenolic content (TPC) levels were correlated with α -amylase inhibitory activity (IC_{50}) in white dragon fruit yogurt, red dragon fruit yogurt and plain yogurt ($R^2 = 0.314, 0.334, \text{ and } 0.457$ respectively). These suggest that the α -amylase inhibitory activity could be attributed by high phenolic content in both dragon fruits.

McCue and Shetty (2004) reported that phenolic compounds tend to bind to the reactive sites of enzymes and alter its catalytic activity. Previous studies suggested that the mechanism of inhibition of the glycolytic activity of α -amylase may occur through the direct blockage of the active center (Randhir and Shetty, 2006) or at several sub sites of the enzyme as also suggested for other plant-based inhibitors (McCue and Shetty, 2004; Payan, 2004).

Furthermore, there are other factors than total phenolics which may play a role in the α -amylase inhibitory activity such as the spectrum of phenolics that are mobilized by thermal processing (Randhir & Shetty, 2006).

5.3 Effects of dragon fruits on proteolysis of yogurt

Protein is an essential nutrient that play important role in healthy growth and maintenance of the body. Milk is one of the most excellent sources of protein that have a good quality of protein content because it contains all the essential amino acids that the body requires (Nurfarah, 2009). However, these milk protein need to be properly digested to avoid problems associated with malabsorption of milk protein (Adibi *et al.*, 2009; Almon *et al.*, 2010; Borchgrevink *et al.*, 2010). Microbial fermentation can increase the digestion of milk and this occurred via proteolytic actions of exogenously excreted microbial enzymes (Korhonen, 2009).

In the present studies, the OPA-based spectrophotometric assay was used showed increased presence α -amino groups resulting from the proteolysis of milk proteins, thus giving a direct measurement of proteolytic activity (Shihata & Shah, 2000). Dragon fruit yogurts at all concentration tested showed no significant different in OPA values compared to plain yogurt ($p=0.061$) (Fig 4.17). These indicate that the addition of dragon fruit into yogurt did not change the proteolysis of milk protein.

Dragon fruit are not significant contributor to yogurt protein because of the low protein content (0.02% w/fresh weight; Ming *et al.*, 1997). Changes in yogurt bacteria growth due to the presence of dragon fruit were not determined and this should be carried out in future studies to confirm minimal effect of dragon fruit on bacterial growth. This is because an increase in OPA values would only occur due to increased bacterial growth. High

population of bacteria normally results in high demand for amino acids which necessitate increase rate of proteolysis (Shah and Shihata, 2000).

5.4. Effects of dragon fruits on exopolysaccharides production of yogurt

Milk products fermented with EPS producing cultures have been reported to show a higher viscosity and a lower degree of syneresis (whey separation) compared with products produced with non-EPS producing cultures (Cerning, 1990; Marshall & Rawson, 1999). This can be explained by the fact that the presence of EPS often imparts a ropy character to the fermented product (Cerning, 1990).

The EPS concentration in yogurt (determined from regression equation of glucose calibration curve ($y=0.348x + 0.069$, $r^2=0.939$); (Figure. 4.23)), showed that dragon fruits caused an increment in exopolysaccharides (EPS) concentration ($p<0.05$) in yogurt. The amount of EPS produced in yogurts in the present studies (180-738mg/L) are within the range of EPS produced as reported in previous studies (180-814mg/L, Savadogo *et al.*, 2003; 250-930mg/L, Lin *et al.*, 2007). Lower EPS production (70-130mg/L) was also reported (Cerning *et al.*, 1997). The wide range in EPS produced in yogurt may be attributed to the yogurt bacterial strains used. Different amount of EPS produced in the presence of *H. undatus* and *H. polyrhizus* suggest that glucose content in both dragon fruits are high since glucose is a primary substrate for EPS biosynthesis (Simova *et al.*, 2004). The stimulatory effects of dragon fruits on EPS production may therefore be attributed to high glucose content in dragon fruit which creates more favourable condition for the bacteria culture (De Vuyst *et al.*, 1999).

EPS can be regarded as an important texture modifying resource because it may act as a natural stabilizer for fermented milk products produced *in situ* by the starter culture. The effect of EPS on the structure and properties of the resulting gel network is, however not

easily predicted. The texture of fermented milk products is not necessarily correlated well with the EPS content (Van Marle and Zoon, 1995) and differences in viscosity may even occur between two cultured skimmed milk products with similar EPS concentration and identical EPS repeating unit structures (Faber *et al.*, 1998). It was reported that the amount of EPS produced by the LAB may range from 150 to 600 mg/L, depending on strains under optimal culture conditions (De Vuyst *et al.*, 1999), and that EPS production by *S. thermophilus* in milk is low under conditions in which the pH is not controlled (De Vuyst *et al.*, 1998).

5.5. Effects of dragon fruits on organoleptic properties of yogurt

Yogurt is characterized as a fermented milk product with a refreshing flavor, a smooth viscous gel and a slight sour taste (Bodyfelt *et al.*, 1988). Thus, any attempts to introduce new types of yogurt with promising functional properties must also address these attributes. Stabilizers are often used during the manufacture of yogurt to enhance and maintain certain characteristics. However, excessive use of stabilizers can negatively affect the sensory properties by providing an unnatural flavor attribute or an over-stabilized (gel like) texture and mouth-feel (Lucey, 2004). Yogurts in the present studies were therefore evaluated for appearance, flavor, texture, and overall quality because these sensory properties are good criteria (Hekmat and Reid, 2006) to describe the quality of yogurt produced upon the addition of dragon fruit.

Dragon fruit yogurts scored higher marks for visual appearance, aroma and sweetness than plain yogurt. The better scores for visual appearance may be attributed to the presence of betacyanins which gave red colour to *H. polyrhizus* (Wybraniec *et al.*, 2007) whereas the aromatic flesh of dragon fruits and the sweetness taste (sugar content) may be directly attributed to the dragon fruits (Ming *et al.*, 1997). Both dragon fruit yogurts gave

lower score for sourness. Since acidification of dragon fruit yogurts were not reduced compared to plain yogurt, the low sourness score in these yogurts may be attributed to their sweetness properties. Lower score for body texture for dragon fruit yogurts compared to plain yogurt could be due to additional water content from that came from the dragon fruit flesh (Ming *et al.*, 1997; Ruzainah Ali Jaafar *et al.*, 2007). Thus, even with the addition of powder milk was made to correct the milk solid content, the water activity from dragon fruit flesh appeared to still contribute significantly to the yogurt water texture. There was relatively weak correlation between body texture score and the EPS production in white dragon fruit yogurt, red dragon fruit yogurt and plain yogurt ($r^2=0.141$, 0.189 , and 0.248 respectively). It was suggested that the formation of EPS by the starter and the probiotic cultures may contribute to the prevention of syneresis, and increase in viscosity combined with a better texture (Kailasapathy, 2006). Polysaccharide producing yogurt bacteria produced EPSs which are important determinants of yogurt viscosity and texture (Griffin *et al.*, 1996).

CONCLUSIONS

This present studies showed that *H. polyrhizus* and *H. undatus* are able to enhance the milk fermentation, lactic acid production of yogurt, and syneresis of yogurt. Both dragon fruits also have increased the therapeutic properties of yogurt with regard to antioxidant activities and *in vitro* inhibition of enzymes relevant to diabetes. The production of EPS was also increased by the addition of *H. polyrhizus* and *H. undatus* besides increase in some organoleptic properties.

REFERENCES

- Abubakar, A., Saito, T., Kitazawa, H., Kawai, Y., Itoch, T. (1998). Structural analysis of new antihypertensive peptides derived from cheese whey protein by proteinase K digestion. *Journal of Dairy Science*, 81, 3131–3138.
- Adolfsson, O., Meydani, S.M., Russell, R.M. (2004). Yogurt and gut function. *American Journal of Clinical Nutrition*, 80, 245-256
- Adolphi, B., Scholz-Ahrens, K.E., De Vrese, M., Açı, Y., Lauer, C., Schrezenmeir, J. (2008). Short-term effect of bedtime consumption of fermented milk supplemented with calcium, inulin-type fructans and caseinphosphopeptides on bone metabolism in healthy, postmenopausal women. *European Journal of Nutrition*, 48, 45-53.
- Ahmad, T., Kanwal, R. (2007). Biochemical characteristics of lactic acid producing bacteria and preparation of camel milk cheese by using starter culture. *Pakistan Veterinary Journal* 24: 279-283.
- Amatayakul, T., Sherkat, F. and Shah, N. P. (2006), Syneresis in set yogurt as affected by EPS starter cultures and levels of solids. *International Journal of Dairy Technology*, 59: 216–221.
- Aryana, K.J., McGrew, P. (2007). Qualities attributes of yogurt with lactobacillus casei and various prebiotics. *Food and Science Technology*, 40, 1808-1814.
- Borchgrevinka, C.P., Elsworth, J.D., Taylor, S.E., Christensen, K.L. (2010) Food intolerances, food allergies, and restaurants. *Journal of Culinary Science & Technology*, 7, 259-284.
- Borges, G.D.S.C., Vieira, F.G.K., Copetti, C., Gonzaga, L.V., Zambiasi, R.C., Filho, J.M., Fett, R. (2011). Chemical characterization, bioactive compounds, and antioxidant capacity of Jussara (*Euterpe edulis*) fruit from the Atlantic Forest in southern Brazil. *Food Research International*, 44, 2128-2133.

- Blum, U. (1998). Effects of microbial utilization of phenolic acids and their phenolic acid breakdown products on allelopathic interactions. *Journal of Chemical Ecology*, 24, 685-708.
- Cai, Y. Z., Luo, Q., Sun, M., & Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sciences*, 74, 2157-2184.
- Cerning, J. (1990). Exocellular polysaccharides produced by lactic acid bacteria. *FEMS Microbiology Letters*, 87, 113-130.
- Cerning, J., Bouillanne, C., Landon M., Desmazeaud M., (1992). Isolation and characterization of exopolysaccharides from slime-forming mesophilic lactic acid bacteria. *Journal of Dairy Science*, 75, 692–699
- Chakraborti, C.K. (2011). The Status of Synbiotics in Colorectal Cancer. *Life Sciences and Medicine Research*, volume 2011.
- Chen, Y., Sun, T., Wang, J., Airden, C., Bai, M., Zhang, H. (2009). Comparison of nutrition and microbiological compositions between two types of fermented milk from Tibet in China. *International Journal of Food Sciences and Nutrition*, 60, 243-250.
- Coisson, J. D., Travaglia, F., Piana, G., Capasso, M., & Arlorio, M. (2005). *Euterpe oleracea* juice as a functional pigment for yogurt. *Food Research International*, 38, 893-897.
- Costa, N.E., O’Callaghan, D.J., Mateo, M.J., Chaurin, V., Castillo, M., Hannon, J.A., McSweeney, P.L.H., Beresford, T.P. (2011). Influence of an exopolysaccharide produced by a starter milk coagulation and curd milk. *International Dairy Journal*, 22, 48-57.
- Damin, M.R., Alcantra, M.R., Nunes, A.P., Oliveira, M.N. (2009). Effects of milk supplementation with skim milk powder, whey protein concentrate and sodium caseinate on acidification, kinetics, rheological properties, and structure of non-fat stirred yogurt. *Food and Science Technology*, 42, 1744-1750.

De Brabandare, A.G., De Baerdemaeker, J.G. (1999). Effects of process conditions on the pH development during yogurt fermentation. *Journal of Food Engineering*, 41, 221-227.

De Vuyst, L.D., Degeest, B. (1999). Heteropolysaccharides from lactic acid bacteria. *FEMS Microbiology Reviews*, 23, 153-177.

De Vuyst, L.D., De Vin, F., Vaningelgem, F, Degeest, B. (2001). Recent developments in the biosynthesis and applications of heteropolysaccharides from lactic acid bacteria. *International Dairy Journal*, 11, 687-707.

De Vuyst, L.D., Weckx, S., Ravyts, F., Herman, L., Leroy, F. (2011). New insight into the exopolisaccharide production of *Streptococcus thermophilus*. *International Dairy Journal*, 21, 586-591.

Dicks, L.M.T., Botes, M. (2010). Probiotic lactic acid bacteria in the gastro-intestinal tract: health benefits, safety and mode of action. *Beneficial Microbes*, 1, 11-29.

Dicks, L.M.T., Silvester, M., Lawson, P.A., Collins, M.D. (2000). *Lactobacillus fornicalis*, isolated from the posterior fornix of the human vagina. *International Journal of Systematic and Evolutionary Microbiology* 50: 1253–1258.

Fliss, I., Fanworth, E. R., Mainville, I., Desjardins, M. P., Gardner, N., Champagne, C. (2007). Growth of probiotic bacteria and bifidobacteria in a soy yogurt formulation. *International journal of Food Microbiology*, 116(1), 174-181.

Fliss, I., Zouhir, A., Kheadr, E., Hamida, J. B. (2011). Partial purification and characterization of two bacteriocin-like inhibitory substances produced by bifidobacteria. *African Journal of Microbiology Research*, 5(4), 411-418.

Fuller, R. (1989). Probiotics in man and animals. *Journal of Applied Bacteriology*, 66, 365–378.

Fuller, R. (1992). History and development of probiotics. In R. Fuller (Ed.), *Probiotics, the Scientific Basis* (pp. 1-8). London, UK: Chapman & Hall.

Gilliland, S. E. (1979). Beneficial inter-relationships between certain microorganisms and humans: Candidate microorganisms for use as dietary adjuncts. *Journal of Food Protection*, 42, 164–167

Gilliland, S. E. (1989). Acidophilus milk products: a review of potential benefits to consumers. *Journal of Dairy Science*, 72, 2483–2494.

Gilliland, S. E., Lara, R. C. (1988). Influence of storage at freezing and subsequent refrigerator temperature on b-galactosidase activity of *Lactobacillus acidophilus*. *Applied Environmental Microbiology*, 45(4), 898–902.

Gilliland, S. E., Nelson, C. R., Maxwell, C. (1985). Assimilation of cholesterol by *Lactobacillus acidophilus*. *Applied and Environmental Microbiology*, 49(49), 377–385.

Gilliland, S. E., Speck, M. L. (1977). Antagonistic action of *Lactobacillus acidophilus* toward intestinal and food-borne pathogens in associative cultures. *Journal of Food Protection*, 40(12), 820–823.

Gilliland, S. E., Speck, M. L. (1977). Deconjugation of bile acids by intestinal lactobacilli. *Applied Environmental Microbiology*, 33(1), 15–18.

Gilliland, S. E., Speck, M. L. (1977). Enumeration and identity of lactobacilli in dietary products. *Journal of Food Protection*, 40(11), 760–762.

Gilliland, S. E., Speck, M. L. (1977). Instability of *Lactobacillus acidophilus* in yogurt. *Journal of Dairy Science*, 60, 1394–1398.

Gilliland, S.E. (2003). Special additional cultures. In: Cogan, T.M. & Accolas, J.P. (eds). *Dairy Starter Cultures*. New York: VCH Publishers, 25-46.

Gobbetti, M., Minervini, F. and Rizzello, C.G. (2004). Angiotensin I-converting-enzyme-inhibitory and antimicrobial bioactive peptide. *International Journal of Dairy Technology*, 57, 172–188.

Goldin, B. R., & Gorbach, S. L. (1984). The effect of milk and *Lactobacillus* feeding on human intestinal bacterial enzyme activity. *American Journal of Clinical Nutrition*, 39, 756-761.

Goldin, B. R., Sherwood, L., Saxelin, M., Barakat, S., Gualteri, L., & Salminen, S. (1992). Survival of *Lactobacillus* species (Strain GG) in human gastrointestinal tract. *Digestive Diseases and Sciences*, 37, 121-128

Gonzalez, N.J., Adikhari, K., Sancho-Madriz, M.F. (2010). Sensory characteristics of peach-flavoured yogurt drinks containing prebiotics and synbiotics. *Food Science and Technology*, 44, 158-163.

Goodno C.C., Swaisgood H.E and Catignani G.L (1981). A fluorimetric assay for available lysine in proteins. *Analytic Biochemistry* 115, pp. 203–211

Granato, D., Branco, G.F., Nazzaro, F., Cruz, A.G., Faria, J.A.F. (2010). Functional foods and nondairy probiotic food development: Trends, concepts, and products. *Comprehensive Reviews in Food Science and Food Safety*, 9, 292–302,

Guache, C., Tomazi, T., Barreto, P.L.M., Ogliari, P.J., Bordignon-Luiz, M.T. (2009). Physical properties of yogurt manufactures with milk whey and transglutaminase. *Food Science and Technology*, 42, 239-243.

Guo, X.H., Kim, M.J., Nam, H.M., Park, S.Y., Kim, J.M. (2010). Screening lactic acid bacteria from swine origins of multistrain probiotics based on in vitro functional properties. *Anaerobe*, 16, 321-326

Gursoy, O., Kinik, O. (2010). Probiotics: A New Popular Option for Cancer Inhibition. *International Journal of Dairy Science*, 5, 241-244.

Hallén, E., Lunden, A., Allmere, T., Andren, A. (2010). Casein retention in curd and loss of casein into whey at chymosin-induced coagulation of milk. *Journal of Dairy Research*, 77, 71-76.

Heck J.M.L., Van Valenberg, H.J.F., Dijkstra, J., van Hooijonk, A.C.M. (2009). Seasonal variation in the Dutch bovine raw milk composition. *Journal of Dairy Science*, 92, 4745-4755

Hekmat, K., Reid, G. (2006). Sensory properties of probiotic yogurt is comparable to standard yogurt. *Nutrition Research*, 26, 163-166.

Hekmat, S., McMahon, D. J. (1992). Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in ice cream for use as a probiotic food. *Journal of Dairy Science* , 75, 1415–1422.

Hoier, E. (1992). Use of probiotic starter cultures in dairy products. *Food Australia*, 44(9), 418–420.

Hosono, A., Kitazawa, H., Yamaguchi, T. (1997). Antimutagenic and antitumour activities of lactic acid bacteria R. Fuller (Ed.), *Probiotics*, vol. 2 Chapman & Hall, UK, 89–132

Jafarei, P., Ebrahimi, M.T. (2011). *Lactobacillus acidophilus* cell structure and applications. *African Journal of Microbiology Research*, 5, 4033-4042.

Jha, A., Mishra, V.K., Mohammad, G. (2008). Immunomodulation and anticancer potentials of yogurt probiotic. *Experimental and Clinical Sciences journal*, 7, 610-618.

Kailasapathy, K. (2006). Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt. *Food and Science Technology*, 39, 1221-1227.

Kailasapathy, K., Phillips, M. (2008). Survival of *Lactobacillus acidophilus* and *Bifidobacterium animalis ssp. Lactis* in stirred fruit yogurts. *Food and Science Technology*, 41, 1317-1322.

Kailasapathy, K., & Rybka, S. (1997). *Lactobacillus acidophilus* and *Bifidobacterium* spp.: Their therapeutic potential and survival in yogurt. *The Australian Journal of Dairy Technology*, 52, 28-35.

Kim, H. S., Gilliland, S. (1983). *Lactobacillus acidophilus* as a dietary adjunct for milk to aid lactose digestion in humans. *Journal of Dairy Science*, 66, 959–966.

Kitazawa H., Nishimura-Uemura, J., Kawai, Y., Otoh, T., Oda, M., Saito, T. (2003). Functional alteration of murine macrophages stimulated with extracellular polysaccharides from *Lactobacillus delbrueckii ssp. bulgaricus* OLL1073R-1. *Food Microbiology*, 20, 267-273.

Kailasapathy, K., Rybka, S. (1997). *L. acidophilus* and *Bifidobacterium* spp. Further therapeutic potential and survival in yogurt. *The Australian Journal of Dairy Technology*, 52, 28–35.

Kamiya, S. (2011). Effect of Probiotics on Intestinal Infections. *Korean Association for the Study of Intestinal Diseases*, 9, 171-178.

Korhonen, H. (2009). Milk-derived bioactive peptides: From science to applications. *Journal of Functional Foods*, 1, 177-187

Korhonen, H., Pihlanto-Leppälä A. (2003). Milk-derived bioactive peptides: Formation and prospects for health promotion. In: C. Shortt and J. O'Brien, Editors, *Handbook of Functional Dairy Products. Functional Foods and Nutraceuticals Series 6.0*, (pp. 109–124) CRC Press, Florida, USA.

H. Korhonen, A. Pihlanto. (2007). Bioactive peptides from food proteins. Y.H. Hui (Ed.), *Handbook of food products manufacturing*, John Wiley and Sons Inc., Hoboken, New Jersey.

H. Korhonen, A. Pihlanto. (2007). Technological options for the production of health-promoting proteins and peptides derived from milk and colostrum. *Current Pharmaceutical Design*, 13 (2007), 829–843

Kumar, V., Rani, A., Dixit, A.K., Pratap, D., Bhatnagar, D. (2009). A comparative assessment of total phenolic content, ferric reducing-anti-oxidative power, free radical-scavenging activity, vitamin C, and isoflavones content in soybean with varying seed coat colour. *Food Research International*, 43, 323-328.

Kurmann, J.A., Rasic, J.L. (1991). The health potential of products containing bifidobacteria. *Applied Food Science*, 1991, 117-157.

Kwon, Y. I., Apostolidis, E., & Shetty, K. (2005). Traditional diet of Americas for management of diabetes and hypertension. *Journal of Medicinal Food*, 10, 266–275.

Kwon, Y. -I., Vatter, D. A., & Shetty, K. (2006). Clonal herbs of Lamiaceae species against diabetes and hypertension. *Asia Pacific Journal of Clinical Nutrition*, 15, 107–118.

Laws, A.P., Leivers, S., Chacon-Romero, M., Chadha. M.J. (2009). Variation in the molecular mass of exopolysaccharides during the time course of extended fermentations of skimmed milk by lactic acid bacteria. *International Dairy Journal*, 19, 768-771.

Lee, D.K., Jang, S., Baek, E.H., Kim, M.J., Lee, K.S., Shin, H.S., Chung, M.J., Kim, J.E., Lee, K.O., Ha, N.J. (2009). Lactic acid bacteria affect serum cholesterol levels, harmful fecal enzyme activity, and fecal water content. *Lipids in Health and Disease*, 8:21

Lee, W.J., Lucey J.A. (2010). Formation and Physical Properties of Yogurt. *Asian-Australian Journal of Animal Science*, 23, 1

Lidbeck, A. (1995). Effect of oral supplementation with lactic acid bacteria during intake of antimicrobial agent. In *International Dairy Lactic Acid Bacteria Conference*, NZ.127-1136.

Lin, J., Lin, Y.-S., Kuo, S.-T., Jiang, C.-M. and Wu, M.-C. (2009), Purification of α -amylase from human saliva by superparamagnetic particles. *Journal of the Science of Food and Agriculture*, 89: 574–578.

Lourens-Hattingh, A., Viljoen, B.C. (2001). Yogurt as probiotic carrier food. *International Dairy Journal*, 11, 1-17.

Mortazavian, A. M., Ehsani, M. R., Mousavi, S. M., Rezaei, K., Sohrabvandi, S., Reinheimer, J. A. (2007), Effect of refrigerated storage temperature on the viability of probiotic micro-organisms in yogurt. *International Journal of Dairy Technology*, 60, 123–127.

McCue, P., & Shetty, K. (2004). Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase *in vitro*. *Asia Pacific Journal of Clinical Nutrition*, 13, 101–106.

Michael, M., Phebus, R.K., Schmidt, K.A. (2010). Impact of a plant extract on the viability of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* in nonfat yogurt. *International Dairy Journal*, 10, 665-672.

Mishra, V.K., Mohammad, G., Jha, A. (2008) Immunomodulation and anticancer potential of yogurt probiotic. *EXCLI Journal*, 7, 177-184

Modler, H. W., McKellar, R. C., Yaguchi, M. (1990). Bifidobacteria and bifidogenic factors. *Canadian Institute of Food Science and Technology Journal*, 23, 29–41.

Nakamura, Y., Yamamoto, N., Skai, K., & Takano, T. (1995). Antihypertensive effect of sour milk and peptides isolated from it that are inhibitors to angiotensin I-converting enzyme. *Journal of Dairy Science*, 78, 1253–1257.

Nielsen, M.S., Martinussen, T., Flambard, M., Sorensen, K.I., Otte, J. (2009). Peptides profile and angiotensin-I-converting enzyme inhibitory activity of fermented milk products: Effect of bacterial strain, fermentation pH and storage time. *International Dairy Journal*, 19, 155-165.

Nomoto, K. (2010). Prevention of infections by probiotics. *Journal of Bioscience and Bioengineering* 100: 583-592.

Novak, L., Loubiere, p. (2000). The metabolic network of *Lactococcus lactis*: distribution of ¹⁴C-labeled substrates between catabolic and anabolic pathways. *J Bacteriol* 182(4), 1136-1143.

Oliveira I, R. B. P., Oliveira I, A. L., & GlóriaI, M. B. A. (2008). Screening of lactic acid bacteria from vacuum packaged beef for antimicrobial activity. *Braz. J. Microbiol.*, 39(2).

Orihara, Y., Furuya, T., Hashimoto, N., Deguchi, Y, Tokoro,K., Kanisawa, T. (1992). Biotransformation of isoeugenol and eugenol by cultured cells of *Eucalyptus perriniana*. *Phytochemistry*, 31, 827-831.

O'Sullivan, M. G., Thornton, G., O'Sullivan, G. C., Collins, J. K. (1992). Probiotic bacteria: myth or reality? *Trends in Food Science and Technology*, 3, 309–314.

Ostlie, H.M., Helland, M.H., Narvhus, J.A. (2003). Growth and metabolism of selected strains of probiotic bacteria in milk. *International Journal of Food Microbiology*, 87, 17-27.

Parra, M. D., Morentin, B. E. M., Cobo, J. M., Lenoir-Wijnkoop, I., Martinez, J. A. (2007). Acute Calcium Assimilation from Fresh or Pasteurized Yoghurt Depending on the Lactose Digestibility Status. *J Am Coll Nutr June 2007*, 26(3), 288-294.

Park, H.G., Wan, H., Kim, S.B., Kim, H.S., Bae, G.S., Chung, S.H., Seo, H.C., Kim, Y.J. (2010). Production of Conjugated Linoleic Acid (CLA) by *Bifidobacterium breve* LMC520 and Its Compatibility with CLA-Producing Rumen Bacteria. *Journal of Agricultural and Food Chemistry*, 59, 984-988.

Park, Y. W., Jin Baek, Y. and Lee, B. H. (2009) Probiotics and Prebiotics as Bioactive Components in Dairy Products, in *Bioactive Components in Milk and Dairy Products* (ed Y. W. Park), Wiley-Blackwell, Oxford, UK.

Proteggente, A.R., Pannala, A.S., Paganga, G., Van Buren, L., Wagner, E., Wiseman, S., Van De Put, F., Dacombe, C., Rice-Evans C.A. (2002). The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radical Research*, 36, 217-233

Rafter, J., Bennett, M., Caderni, G., Clune, Y., Hughes, R., Karlsson, P.C., Klinder, A., O’Riordan, M., O’Sullivan, G.C., Pool-Zobel, B., Rechkemmer, G., Roller, M., Rowland, I., Salvadori, M., Thijs, H., Van Loo, J., Watzl B., Collins, J.K. (2007). Dietary synbiotics reduce cancer risk factors in polypectomized and colon cancer patients. *The American Journal of Clinical Nutrition*, 85, 488-496.

Ramchandran, L., Shah, N.P. (2009). Effect of exopolysaccharides on the proteolytic and angiotensin-I converting enzyme-inhibitory activities and textural and rheological properties of low fat yogurt during refrigerated storage. *Journal of Dairy Science*, 92, 895-906.

Ranadheera, R.D.C.S., Baines, S.K., Adams, M.C. (2009). Importance of food in probiotic efficacy. *Food Research International*, 43, 1-7.

Randhir R and Shetty K (2006). Mung beans processed by solid-state bioconversion improves phenolic content and functionality relevant for diabetes and ulcer management, *Innovative Food Science & Emerging Technologies* 8, pp. 197–204.

Randhir R, Shetty K. (2007). Improved alpha-amylase and *Helicobacter pylori* inhibition by fenugreek extracts derived via solid-state bioconversion using *Rhizopus oligosporus*. *Asia Pacific Journal of Clinical Nutrition*, 16, 382-92.

Ranilla, L.G., Kwon, Y.I., Apostolidis, E., Shetty, K. (2010). Phenolic compounds, antioxidant activity and in vitro inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. *Bioresource Technology*, 101, 4676-4689.

Rasic, J. L., & Kurman, J. A. (1978). Yoghurt-scientific grounds, technology, manufacture and preparations. Copenhagen: Technical Dairy Publishing House.

Rasic, J. L., Kurmann, J. A. (1983). Bifidobacteria and their role (pp. 12,13, 21, 42–50, 102–133, 144–158). Basel: Birkhauser.

Rutherford, S.M. (2010). Methodology for determining degree of hydrolysis of proteins in Hydrolysates: a review. *US National Library of Medicine National Institute*, 93, 1515-1522.

Rybka S and Kailasapathy K (1995). The survival of culture bacteria in fresh and freeze-dried AB yoghurts, *Australian Journal of Dairy Technology* 50, pp. 51–57.

Rybka, S. (1994). The enumeration of *Lactobacillus*, *Streptococcus* and *Bifidobacterium* species in yogurt. *B.Sc. dissertation, University of New South Wales, Sydney*.

Sanchez, B., De Los Reyes-Gavilan, C. G., Margolles, A. and Gueimonde, M. (2009). Probiotic fermented milks: Present and future. *International Journal of Dairy Technology*, 62, 472–483.

Sanders, M.E. (2009). How do we know when something called “probiotic” Is eally a robiotic? A guideline for consumers and health care professionals. *Functional Food Reviews*, 1, 1

Sanders, M.E. (1997). Lactic acid bacteria as promoters for human health. In: *Functional Foods*. Eds. Goldberg, L. Chapman and Hall Co., New York, pp. 294-322.

Sanders, M.E., Klaenhammer, T.R. (2001). Invited review: the scientific basis of *Lactobacillus acidophilus* NCFM functionality as a probiotic. *Journal of Dairy Science* 84: 319–331.

Sandine, W. E. (1979). Role of Lactobacillus in the intestinal tract. *Journal of Food Protection*, 42, 295

Savadogo, A., OuattaraCheik, A. T., Bassole Imael, H. N., & Traoret, S. A. (2006). Bacteriocins and lactic acid bacteria - a minireview. *African Journal of Biotechnology*, 5(9), 678-683.

Savadogo, A., Ouattara, A. T., Bassole, H. N., & Traore, A. S. (2004). Antimicrobial activities of lactic acid bacteria strains isolated from Burkina Faso fermented milk. *Pakistan Journal of Nutrition*, 3(3), 174-179.

Serra, M., Trujillo, A.J., Guamis, B., Ferragut, V. (2009). Proteolysis of yogurts made from ultra-high-pressure homogenized milk during cold storage. *Journal of Dairy Science*, 92,71-78

Shaaban, M.A.E., Abo sree, Y.H., Bayoum, H.M., Eissa, H.A. (2010). The use of lemongrass extracts as antimicrobial and food additive potential in yogurt. *Journal of American Science*, 6, 582-594.

Shah, N.P. (2000). Effect of milk-derived bioactives: an overview. *British Journal of Nutrition*, 84, 3-10.

Shah, N.P. (2000). Probiotic bacteria: Selective enumeration and survival in dairy foods. *Journal of Dairy Science* 83, 894-907

Shah, N.P. (2007). Functional cultures and health benefits. *International Dairy Journal*, 17, 1262–1277

Shah, N.P., Dave, R.I. (1995). Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures, *Int. Dairy Journal* 7, 31-41

Shah, N. P., Lankaputhra, E. V. (1997). Improving viability of *Lactobacillus acidophilus* and *Bifidobacterium spp.* in yogurt. *International Dairy Journal*, 7, 349–356.

Shaukat, A., Levitt, M. D., Taylor, B.C., MacDonald, R., Shamliyan, T. A., Kane, R. L., Wilt, T. J. (2010). Systematic Review: Effective Management Strategies for Lactose Intolerance. *Ann Intern Med*, 152(12), 797-803.

Shetty, K. (1997). Biotechnology to harness the benefits of dietary phenolics; focus on *Lamiaceae*, *Asia Pacific Journal of Clinical Nutrition* 6, 162-171

Shetty, K., Apostolidis, E. & Kwon, Y.I. (2006). Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension *Innovative Food Science and Emerging Technologies* 8, 46–54.

Shetty, K., Clydesdale, F. and Vatter, D. (2005). Clonal screening and sprout based bioprocessing of phenolic phytochemicals for functional foods. In: K.Shetty, G.paliyath, A.Pometto and R.E.Levin, Editors, *Food biotechnology* (2nd edition), CRC Taylor & Francis, New York, 603.

Shihata, A., Shah, N. P. (2000). Proteolytic profiles of yogurt and probiotic bacteria. *International Dairy Journal* 10, 401-408.

Shihata, A., Shah, N.P. (2002) Influence of addition of proteolytic strains of *Lactobacillus delbrueckii subsp. bulgaricus* to commercial ABT starter cultures on texture of yoghurt, exopolysaccharide production and survival of bacteria. *International Dairy Journal*, 12, 765-772.

Simova, E., Frengova, G. and Beshkova, D. (2004), Exopolysaccharides produced by mixed culture of yeast *Rhodotorula rubra* GED10 and yogurt bacteria (*Streptococcus thermophilus* 13a + *Lactobacillus bulgaricus* 2-11). *Journal of Applied Microbiology*, 97, 512–519.

Soghomonyan, D., Akopyan, K., Trchounian, A. (2009). pH and oxidation-reduction potential change of environment during growth of lactic acid bacteria: Effects of oxidizers and reducers. *Applied Biochemistry and Microbiology*, 47, 27-31.

Sovova, M., Sova, P., Mrazova, A. (2002). Pharmaceutical importance of *Allium sativum* L. 3. Antibacterial effects on *Helicobacter pylori*. *Ceska a Slovenska Farmacie : Casopis Ceske Farmaceuticke Spolecnosti a Slovenske Farmaceuticke Spolecnosti*, 51(4), 168-172.

Tamine, A. Y., & Deeth, H. C. (1980). Yogurt: technology and biochemistry. *J Food Prot*, 43(12), 939-977.

Tamine, A.Y, Hassan, A., Farnworth, E. and Toba, T. (2007) Structure of Fermented Milks, in: Tamime, A, Editor, *Structure of Dairy Products*, Blackwell Publishing Ltd, Oxford, UK.

Tamine A.Y, Kalab M, Davies G, Mahdi HA (1991). Microstructure and firmness of labneh (high solid yoghurt) made from cow's, goat's and sheep's milk by a traditional method or by ultrafiltration. *Food Structure* 10, 37-44.

Tamine, A. Y., Marshall, V. M. E., a Robinson, R. K. (1995). Microbiological and technological aspects of milks fermented by bifidobacteria. *Journal of Dairy Research*, 62, 151–187.

Tiwari, A., & Rao, K. (2002). Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Current Science*, 83, 30–38. • A.Y.

Tamine, A. Y., Robinson, R. K. (1985). Yogurt science and technology. *Pergamon Press, New York, NY*.

Tamime, R.K. Robinson. Biochemistry of fermentation. A.Y. Tamime, R.K. Robinson (Eds.), *Yoghurt: Science and Technology*, Pergamon, Oxford, England (1999), pp. 433–48

Vaclavik, V. A., Christian E. W. (2008) Milk and Milk Products. Rankin, S (Ed.) Essentials of Food Science. *John Wiley and Sons Inc., Hoboken, New Jersey*.

Valente, A., Albuquerque, T.G., Sanches-Silva, A., Costa, H.S. (2011). Ascorbic acid content in exotic fruits: A contribution to produce quality data for food composition databases. *Food Research International*, 44, 2237-2242.

Vargas, M., Chafer, M., Albors, A., Chiralt, A., Gonzalez-Martinez, C. (2008). *International Dairy Journal*, 18, 1146-1152.

Velioglu, Y. S., Mazza, G., Gao, Y. L., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *Journal of Agricultural and Food Chemistry*, 46, 4113–4117.

Viljoen, C., Lourens-Hattingh, A., and Bennie (2001). Yogurt as probiotic carrier food, *International Dairy Journal* 11, 1–17

Wallace, T. C., Guarner, F., Madsen, K., Cabana, M. D., Gibson, G., Hentges, E. and Sanders, M. E. (2011). Human gut microbiota and its relationship to health and disease. *Nutrition Reviews*, 69, 392–403.

Wang, H., Cao, G. H., & Prior, R. L. (1996). Total antioxidant capacity of fruits. *Journal of Agricultural and Food Chemistry*, 44, 701–705.

Weng, Wei-Lien. 2008. Antimicrobial effect of yogurt lactic acid bacteria and muscadine products on *Enterobacter sakazakii*. *MISSISSIPPI STATE UNIVERSITY*.

Wichienchot, S., Jatupornpipat, M., and Rastall, R.A. (2009). Oligosaccharides of pitaya (dragon fruit) flesh and their prebiotic properties. *Food Chemistry* 120: 850-857.

Wollowski, I., Rechkammer, G., Pool-Zobel, B.L (2001).Protective role of probiotics and prebiotics in colon cancer. *American Journal of Clinical Nutrition*, 73, 451–455

Wynckel, A., *et al*, (1991). Intestinal absorption of calcium from yogurt in lactase-deficient subjects. *Reprod Nutr Dev*. 31:411–418.

Zhang, S., Zhang, L. (2012). Effect of Exopolysaccharide Producing Lactic Acid Bacterial on the Gelation and Texture Properties of Yogurt. *Advance Materials Research* 430-432, 890-893.

Zouhir, A., Kheadr, E., Fliss, I., Ben Hamida, J. (2010). Partial purification and characterization of two bacteriocin-like inhibitory substances produced by *bifidobacteria*. *African Journal of Microbiology Research*, 5, 411-418.

APPENDICES

APPENDIX I

1. Chemicals reagents

i) Preparations of yogurt.

Pasteurized Dutch-lady full cream milk and Dutch-lady full cream milk powder.

ii) Preparation of water extracts from yogurt.

1M Hydrochloric acid (HCL), 0.5M Natrium Hydroxide (NaOH) and distilled water

iii) Total phenolic assay and DPPH antioxidant assay

95% Ethanol (System), 50% Folin-Ciocalteu reagent (Merck), 5% Sodium carbonate, Na_2CO_3 (Merck) MW: 105.99 g/mol, Gallic Acid, $\text{C}_7\text{H}_6\text{O}_5$ (Sigma) FW: 170.12 g/mol, and Methanol, CH_3OH (System) MW: 32.04 g/mol.

iv) OPA assay

O-Pthaldialhyde (OPA), $\text{C}_8\text{H}_6\text{O}_2$ (Sigma) FW: 134.13 g/mol, Sodium tetraborate, 99.5-105% $\text{B}_4\text{Na}_2\text{O}_7$ (Sigma) FW: 288.38 g/mol, Methanol, CH_3OH (System) MW: 32.04 g/mol, β -mercaptoethanol, $\text{C}_2\text{H}_6\text{O}_5$ (Sigma) FW: 78.13 g/mol and Tryptone (Sigma).

v) α -Amylase inhibitory assay

Porcine pancreatic alpha-amylase (Sigma 3176) Type VI-B, 10-30 units/ mg solids containe lactose, 3,5-dinitrosalicylic acid, $\text{C}_7\text{H}_4\text{N}_2\text{O}_7$ (Sigma) FW: 228.12 g/mol, Potassium-sodium tartarate-4-hydrate, $\text{C}_4\text{H}_4\text{KNaO}_6 \cdot 4\text{H}_2\text{O}$ (System) FW : 282.23 g/mol, Starch soluble, $(\text{C}_6\text{H}_{10}\text{O}_5)_n$ (system).

vi) α -Glucosidase inhibitory assay

α -glucosidase, Type 1, baker Yeast (Sigma G5003), 1KU, 175.44 mg solids, P-nitrophenyl- α -D-glucopyranoside, C₁₂H₅NO₈ (Sigma) FW: 301.25 g/mol, Di-potassium hydrogen phosphate, K₂HPO₄ (System) FW: 174.18 g/mol, Di-potassiumdihydrogen phosphate, KH₂PO₄ (Merck) FW: 136.09 g/mol.

2. Equipments

pH meter (Cyperscan510), Ice-maker (Scotsman AF 200), Autoclave machine (Tomy Autoclaves), Oven (Memert), Centrifuge Machine (Eppendorf 5804R), Water bath (Julabo, Model Sw-21C, Haake Model SWD 20, 4°C refrigerator (National Green Earth Fridge NR-B72FB), -20°C Refrigerator (Sanyo Ultra Low MDFU 4086S), Spectrophotometer, (Shimadzu UV mini 1240), and Weighing Machine (Adventure Ohaus)

APPENDIX II

1. OPA values for plain and dragon fruit yogurts

| SAMPLES | PEPTIDE CONCENTRATIONS (mg/ml) |
|---------|--------------------------------------|
| P | 31.53 ± 0.02 |
| W10 | 31.67 ± 0.04 |
| W20 | 31.63 ± 0.01 |
| W30 | 31.82 ± 0.02 |
| R10 | 31.62 ± 0.03 |
| R10 | 31.85 ± 0.05 |
| R30 | 31.76 ± 0.03 |

2. Total phenolic content in yogurts

| SAMPLES | PHENOLIC CONTENT (ugGAE/ml) |
|---------|--------------------------------|
| P | 20.25 ± 0.01 |
| W10 | 36.44 ± 0.04 |
| W20 | 54.34 ± 0.05 |
| W30 | 64.43 ± 0.05 |
| R20 | 37.16 ± 0.03 |
| R10 | 43.22 ± 0.02 |

| | |
|-----|------------------|
| R30 | 49.61 \pm 0.03 |
|-----|------------------|

3. Yogurts syneresis

| SAMPLES | % SYNERESIS |
|---------|------------------|
| P | 52.93 \pm 0.01 |
| W10 | 57.19 \pm 0.05 |
| W20 | 56.57 \pm 0.05 |
| W30 | 63.16 \pm 0.03 |
| R10 | 57.76 \pm 0.03 |
| R20 | 61.24 \pm 0.02 |
| R30 | 70.32 \pm 0.02 |

4. DPPH antioxidant assay

| SAMPLES | % INHIBITION |
|---------|------------------|
| P | 19.16 \pm 0.03 |
| W10 | 24.97 \pm 0.05 |
| W20 | 33.60 \pm 0.03 |
| W30 | 39.96 \pm 0.02 |
| R10 | 33.71 \pm 0.01 |
| R20 | 42.26 \pm 0.03 |
| R30 | 45.74 \pm 0.03 |

5. Inhibition of α -amylase activity

| SAMPLES | % INHIBITION |
|---------|------------------|
| P | 19.70 \pm 0.07 |
| W10 | 17.30 \pm 0.09 |
| W20 | 27.10 \pm 0.03 |
| W30 | 44.65 \pm 0.04 |
| R10 | 22.20 \pm 0.03 |
| R20 | 38.40 \pm 0.05 |
| R30 | 52.20 \pm 0.04 |

| SAMPLES | IC ₅₀ (mg/g) |
|---------|-------------------------|
| P | 70.18 \pm 0.03 |
| W10 | 74.37 \pm 0.05 |
| W20 | 48.92 \pm 0.03 |
| W30 | 27.60 \pm 0.06 |
| R10 | 62.31 \pm 0.05 |
| R20 | 33.2 \pm 0.05 |
| R30 | 24.79 \pm 0.04 |

6. Inhibition of α -glucosidase activity

| SAMPLES | % INHIBITION |
|---------|------------------|
| P | 9.21 \pm 0.07 |
| W10 | 8.70 \pm 0.09 |
| W20 | 19.79 \pm 0.03 |
| W30 | 37.10 \pm 0.04 |
| R10 | 15.69 \pm 0.05 |
| R20 | 27.38 \pm 0.04 |
| R30 | 34.02 \pm 0.09 |

| SAMPLES | IC ₅₀ (mg/g) |
|---------|-------------------------|
| P | 143.47 \pm 0.08 |
| W10 | 147.98 \pm 0.09 |
| W20 | 69.34 \pm 0.03 |
| W30 | 33.67 \pm 0.02 |
| R10 | 82.46 \pm 0.05 |
| R20 | 48.13 \pm 0.03 |
| R30 | 36.43 \pm 0.04 |

7. Exopolysaccharides production of yogurts

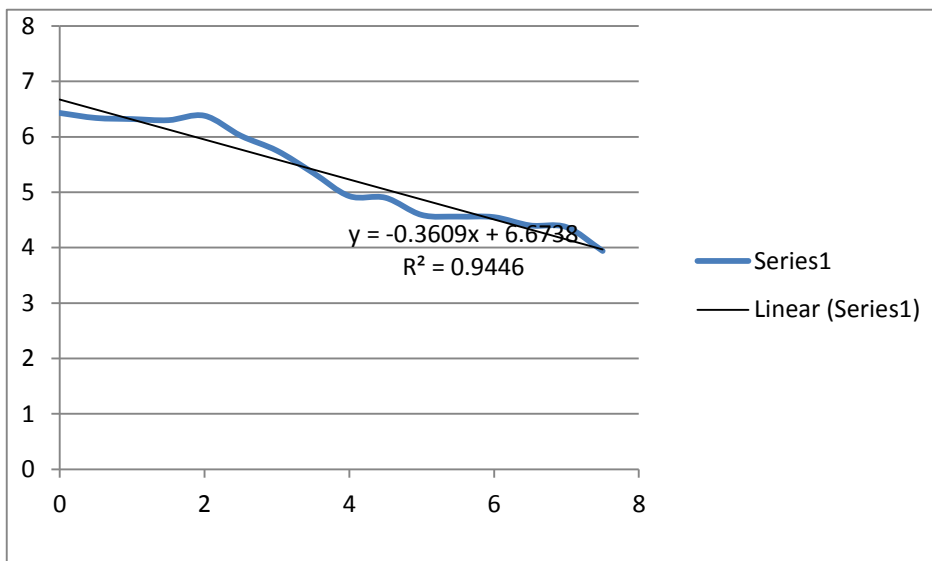
| SAMPLES | EXOPOLYSACCHARIDES CONCENTRATION (mg/L) |
|---------|--|
| P | 181 \pm 0.05 |
| W10 | 288 \pm 0.04 |
| W20 | 474 \pm 0.03 |
| W30 | 713 \pm 0.05 |
| R20 | 214 \pm 0.06 |
| R10 | 549 \pm 0.04 |
| R30 | 738 \pm 0.03 |

8. Sensory evaluation of plain and dragon fruit yogurts

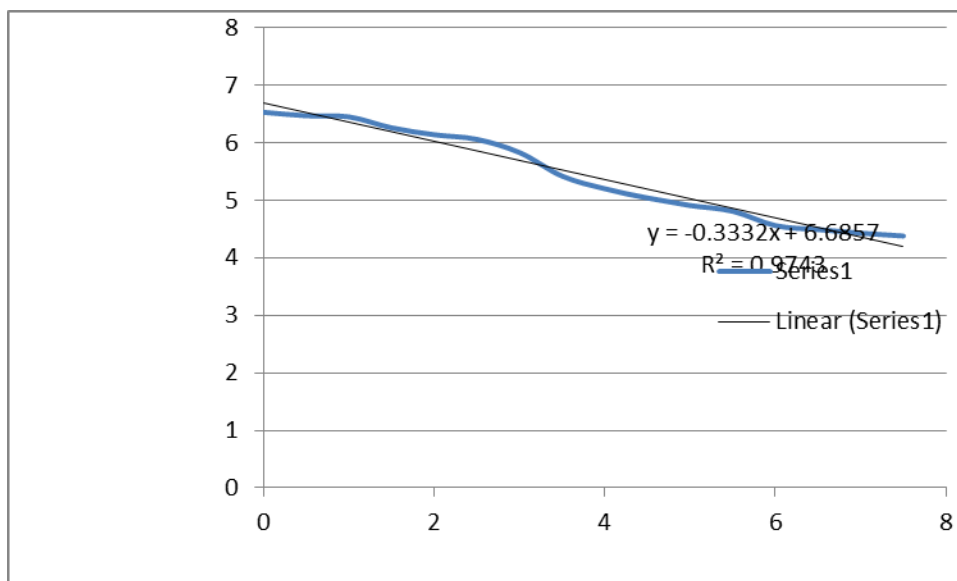
| Samples | VA | BT | A | SW | SO | OT |
|---------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| R | 7.77 \pm 0.05 | 5.90 \pm 0.06 | 5.90 \pm 0.05 | 4.22 \pm 0.02 | 5.27 \pm 0.07 | 4.95 \pm 0.04 |
| W | 5.81 \pm 0.08 | 5.95 \pm 0.04 | 5.32 \pm 0.03 | 3.91 \pm 0.04 | 6.09 \pm 0.04 | 5.18 \pm 0.04 |
| P | 6.90 \pm 0.04 | 6.81 \pm 0.03 | 5.59 \pm 0.03 | 3.43 \pm 0.05 | 7.13 \pm 0.05 | 5.45 \pm 0.06 |

9. Milk fermentation rate determination

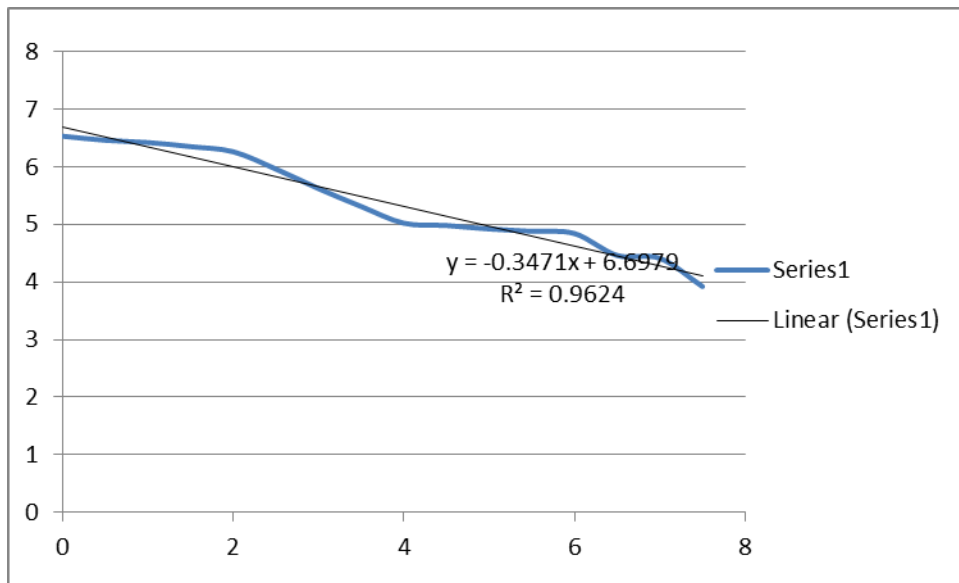
W10



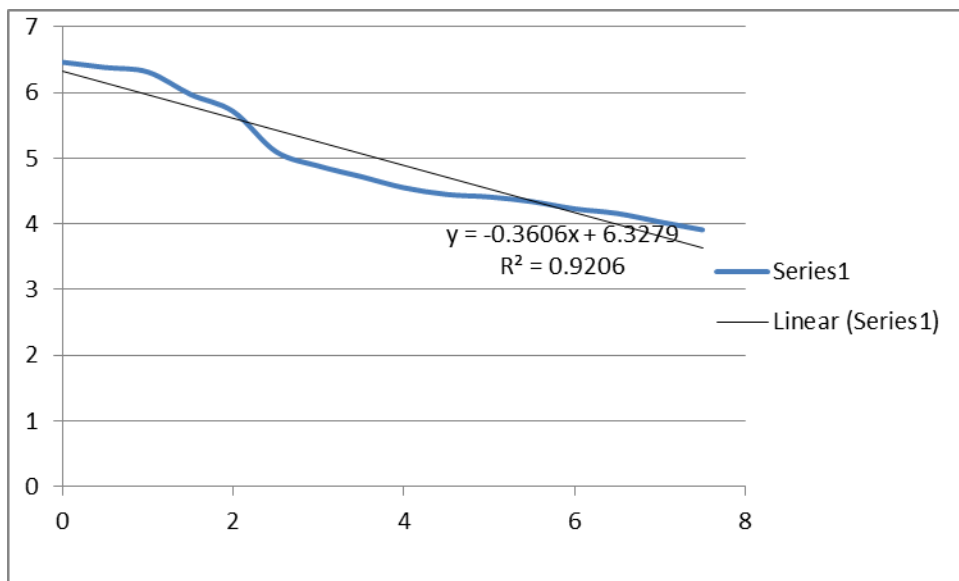
W20



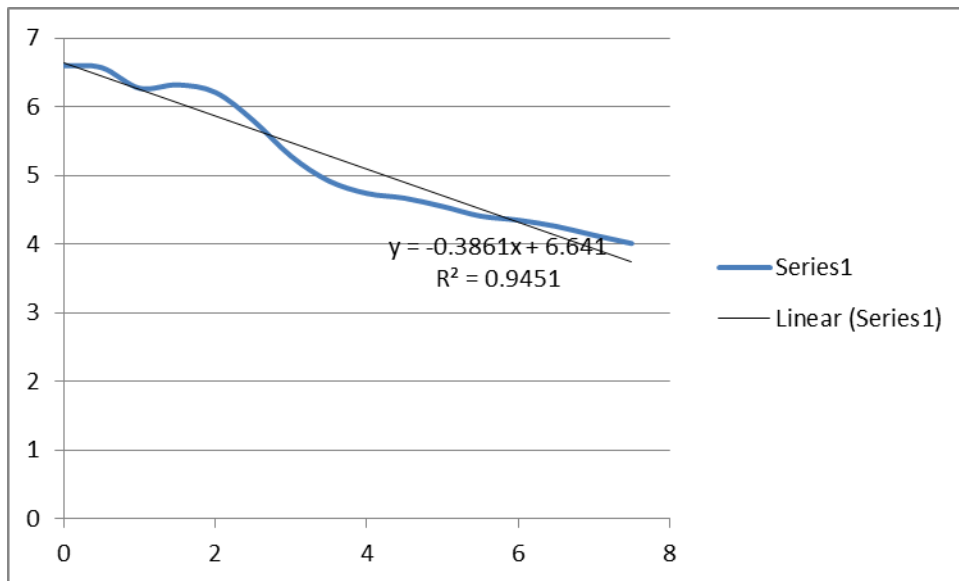
W30



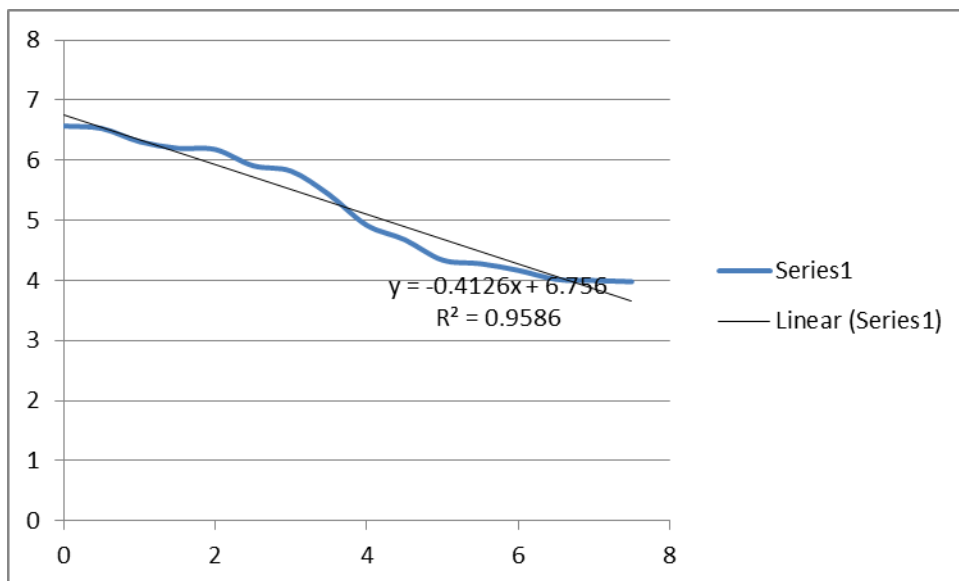
PLAIN



R10



R20



R30

